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CYTOGENETIC ANALYSIS OF HUMAN CHROMOSOMES AND ITS VALUE FOR THE ESTIMATION OF GENETIC RISK

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Summary

A short review of present-day contradictory opinions on the usefulness of human chromosomal analysis in the system of chemical mutagen testing is illustrated by examples of the results achieved by both conventional and banding techniques. The results include exposures of human chromosomes to ECHH and TEPA in vitro, and to ECHH, vinyl chloride and imuran in vivo. Exposures of human lymphocytes in vitro to the chemical to be tested for mutagenicity are recommended as one of the tests to be included in the system of mutagenicity testing, parallel with all other tests on mammalian and submammalian levels. The testing of human chromosomes of people exposed to chemicals in vivo is considered essential.

Introduction

What is the actual place of cytogenetic analysis of human chromosomes in the system of chemical mutagen testing? There are a number of contradictory opinions ranging from a complete rejection of this method, particularly for routine testing, to its acceptance as a unique technique for the estimation of genetic risk. The cytogenetic methods suitable for the analysis of human chromosomes are well established, widespread and not too expensive, so that they could be used for large scale routine population studies as well as for systematic experiments in vitro. Nevertheless, it is frequently objected that the chromosomal aberrations detected represent only a part – and maybe the less important one – of all genetic changes induced by mutagens. Cytogenetic methods cannot, for example, detect point mutations. However, the factors that induce chromosomal aberrations usually cause point mutations as well [15–18].

Another objection argues that human peripheral lymphocytes used for testing, in vitro and in vivo, can yield only an average level of chromosome changes occurring in somatic cells whereas for future generations it is more important to control the chromosome changes that occur in gonadal cells.

The detection of chromosome changes in somatic cells could be valuable from the aspect of carcinogenicity: many mutagens are also carcinogens and there is a hypothetical relationship between chromosomal aberrations and the origin of tumours [2]. It can also be logically assumed that the chemical that is harmful to somatic cell chromosomes is also harmful to chromosomes in gonads, even though on a different level.

Quite a serious criticism has also been raised against scoring chromosome changes in human cells exposed in vitro. It is true that experimental conditions in vitro are different from those in living organisms, where most probably each chemical substance is subject to metabolic and biochemical changes. If the cells are exposed in vitro, these biochemical changes are absent so that we get a simplified but rather artificial situation. In an attempt to avoid this discrepancy, the method using pre-activation of the chemical in some biological system before the experimental application is under discussion [20,18].

Another problem is that many chemicals are cell-stage specific in their activity [1,11]. Cytogenetic analysis of lymphocytes from exposed persons in vivo may reveal their mutagenic effect provided that G_1 stage cells are sensitive to the particular chemical. On the other hand, in experiments with human lymphocytes exposed to chemicals in vitro, we can test the sensitivity of cells in all stages of the cell cycle.

Our laboratory is interested in all these problems and that is why we have undertaken a series of mutagenic experiments with human lymphocytes using different applications and cytogenetic techniques as well as different approaches to the problems. Our objective is to find out the real value of cytogenetic analysis of human cells for the whole mutagenicity testing system.

Material and methods

A short-term cultivation (56—58 h) of peripheral human lymphocytes [9] was used in all our cytogenetic studies. Slides were scored blindly after coding. For a detailed analysis, mentioned in the Discussion, the trypsin-banding technique [3] was also used. The results were mathematically evaluated using the χ^2 test.

Results and discussion

Looking for a model chemical substance for our complex cytogenetic study we chose epichlorohydrin (ECHH) (1-chloro-2,3-epoxy-propane), a bi-functional alkylating agent with potential mutagenic effect that until recently has not been sufficiently tested. There were two reasons for this choice. The first was that this chemical is used in our chemical industry as a solvent and as a basic compound for the production of epoxy resins, so that large groups of workers are exposed to it. The second reason was linked with our opportunity

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The experiments started with an informative study concerned with exposures of human lymphocytes in vitro. In these experiments the effect of ECHH was compared with the effect of another alkylating agent TEPA (tris (1-aziridinyl)phosphine oxide) that is recognized as a strong mutagen. We have found that ECHH is a 4—5 times less effective mutagen that TEPA [12] but its effectivness is dose dependent.

The next step in this study was provoked by the question whether there are some undetected chromosomal aberrations if only a conventional cytogenetic technique is used. That is why we also used the trypsin-banding technique [3] in our experiments and compared both results. Our comparative analysis of chromosomal aberrations caused by ECHH and TEPA in vitro resulted in a conclusion that for large cytogenetic population studies it is not necessary to use banding techniques, because only a negligible fraction of all chromosome aberrations escapes detection by conventional techniques [13].

However, the banding technique enables us to detect the distribution of breaks on individual chromosomes and their parts. There was detected a highly significant non-randomness of break distribution caused by ECHH and TEPA in vitro in our material [13].

A newly started production of ECHH in one of our chemical plants has allowed us to initiate a prospective long-term study of workers with ECHH exposure in vivo. Peripheral blood samples taken from these workers even before they started to work in the plant served as their own control. Another chromosomal analysis of blood samples was repeated after one year's exposure and we intend to repeat it 2 or 3 times after at least one year's interval.

This study is being done in co-operation with the Laboratory of Mutagenesis of the Institute of Medical Genetics of the Academy of Medical Sciences in Moscow, where half of the all material is scored. The first results of our part of this prospective study are shown in Table I.

A significant increase of aberrant cells was found in peripheral lymphocytes of occupationally exposed workers after one year's exposure to ECHH. The study continues and the final results will be published later.

For the past two years, the mutagenic activity of ECHH has also been tested by other workers in our institute using different mutagenicity tests [19] (Table II). Most of these results show the mutagenic effect of ECHH and are in agreement with results of our cytogenetic study in vivo.

Since the prospective type of study in men in vivo is laborious and time consuming we decided to try an opposite and more common approach, i.e. to collect and analyse blood samples from workers who had already been exposed to a mutagen for a long time, at least for 10 years. For this informative study we chose nine workers with long-term exposure to vinyl chloride, which is known for its carcinogenic and mutagenic effects [4,7,14]. The results of two cytogenetic analyses conducted in each worker of the group are presented in Table III. The time between the first and second analysis was 8 months. It was interesting that in some workers we detected a very high increase of aberrant cells while in others the number of these cells remained at the control levels. Non-homogeneity of cytogenetic results found in this group by the first and

TABLE I

LONG-TERM STUDY OF WORKERS EXPOSED TO EPICHLOROHYDRIN

		Number of cells scored	Cells with aberrations		Types of aberration				Number of aberrations per 100 cells	Number of breaks per 100 cells	Number of gaps
			Number	%	Number of chromatid breaks	Number of chromatid exchanges	Number of chromosomal breaks	Number of chromosomal exchanges			
Workers with ECHH	1	3 573	41	1.2	31	1	7	4	1.2	1.3	14
	2	3 336	67	2.0 [a]	34	5	21	7	2.0	2.4	19

1, Before exposure, controls.
2, After one year of exposure.
[a] Significantly higher than in controls ($P < 0.01$).

TABLE II

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TABLE II

TESTING OF MUTAGENIC ACTIVITY OF ECHOL (1974–1976)

Test	Testing system	Results
Test on microorganism [19]	*Salmonella typhimurium*	+ D
Host-mediated assay [19]	*Salmonella typhimurium*	+ D
Cytogenetic analysis of bone-marrow cells of mammals [19]	Mouse	+ D
	Chinese hamster	+ D
Dominant-lethal test [19]	Mouse	–
Human lymphocytes exposure in vitro [12]	Peripheral lymphocytes	+ D
Human lymphocytes, exposure in vivo	Peripheral lymphocytes	+

+, Mutagenic activity.
D, Dose dependence.

also by the second analysis was unusual in our material. The increase of aberrant cells detected by the second analysis occurred in lymphocytes of some workers who showed no cytogenetic changes at the time of the first analysis, and there was no time-dependent effect of exposure. We hypothetically ascribe this observation to an irregular exposure due the cyclic character of the production process.

The results of another cytogenetic study of persons exposed to chemicals in vivo demonstrate a dose-dependent and time-dependent increase of chromosomal aberrations in the chronically exposed persons. This time the chromosomes of patients treated with cytostatic Imuran were analysed (Table IV). The upper part of this table reports four nephrotic patients treated with Imuran for 6 months up to 2 years, the lower part of Table IV describes four patients with kidney transplantation because of glomerulonephritis, who were treated for 6 months up to 7 years. The literature data published on the effect of Imuran are rather contradictory as to its mutagenic action on human chromosomes in vivo [5–7,10]. Our results seem to indicate that Imuran has a time-dependent, and probably also dose-dependent, effect on human lymphocytes because all the patients treated with this drug for 3 and more years without interruption showed a significantly increased number of cells with aberrations. These findings can also explain the negative results found by some authors in patients treated with Imuran for a shorter period.

Conclusions

What is our opinion on the use of human cells exposed to chemicals in vitro and in vivo and its importance for the system of mutagenicity testing? Present cytogenetic experiences lead us to the conclusion that experiments in vitro provide a unique opportunity to detect directly the sensitivity of human cells to the chemical tested. Especially dose dependence could be tested easily. We believe that these tests should be included if possible in the routine testing system parallel with all other tests on mammalian and sub-mammalian levels, and if so, the pre-activation of the chemical to be tested for mutagenicity should be preferred.

The testing of chromosome changes in lymphocytes of persons already

TABLE III

CHROMOSOMAL ANALYSIS OF WORKERS PROFESSIONALLY EXPOSED TO VINYL CHLORIDE

Patient	Number of cells scored	Cells with aberrations		Types of aberrations					Number of aberrations per 100 cells	Number of breaks per 100 cells	Number of gaps	Exposure (years)
		Number	%	Number of chromatid breaks	Number of chromatid exchanges	Number of chromosomal breaks	Number of chromosomal exchanges	Number of despiralizations				
E.H.	100	11	11.0	5	1	4	0	1	11	11	4	10 (5 [a] + 5 [b])
	100	0	0	0	0	0	0	0	0	0	0	10 + 8 months
A.T.	100	4	4.0	2	0	1	0	1	4	3	1	9 [b]
	100	1	1.0	0	0	1	0	0	1	1	0	9 + 8 months
J.P.	100	4	4.0	4	0	0	0	0	4	4	0	16 [a]
	100	2	2.0	0	0	1	1	0	2	3	0	16 + 8 months
E.J.	100	3	3.0	1	1	0	1	0	3	5	0	12 [a]
	100	6	6.0	4	0	2	0	0	6	6	2	12 + 8 months
K.K.	100	2	2.0	2	0	0	0	0	2	2	2	15 [a]
	100	7	7.0	6	1	0	0	0	7	8	2	15 + 8 months
F.M.	100	1	1.0	1	0	0	0	0	1	1	1	11 [a]
	100	2	2.0	1	0	1	0	0	2	2	0	11 + 8 months
A.H.	100	1	1.0	1	0	0	0	0	1	1	0	14 [b]
	100	1	1.0	1	0	0	0	0	1	2	0	14 + 8 months
J.K.	100	1	1.0	0	0	1	0	0	1	1	0	16 [b]
	100	4	4.0	3	0	1	0	0	4	4	2	16 + 8 months
M.M.	69	0	0	0	0	0	0	0	0	0	1	12 [b]
	100	1	1.0	0	0	0	1	0	1	2	0	12 + 8 months
Controls	3573	41	1.2	31	1	7	4	0	1.2	1.3	14	

[a] High exposure, more than 500 p.p.m.
[b] Exposure 500 p.p.m.

TABLE IV

CHROMOSOMAL ABERRATIONS AFTER EXPOSURE TO [illegible]ORAN IN VIVO

	100	1	1.0	0	0	1	0	1	2	0	1	months	
Controls	3573	41	1.2	31	1	4	0	1.2	1.3	14			

[a] High exposure, more than 500 p.p.m.
[b] Exposure 500 p.p.m.

TABLE IV

CHROMOSOMAL ABERRATIONS AFTER EXPOSURE TO IMURAN IN VIVO

Patient	Age (years)	Years of treatment (dose per day)	Number of cells scored	Cells with aberrations		Types of aberration				Number of despiralizations	Number of aberrations per 100 cells	Number of breaks per 100 cells	Number of gaps
				Number	%	Number of chromatid breaks	Number of chromatid exchanges	Number of chromosomal breaks	Number of chromosomal exchanges				
A.S.	1	0.5 (25 mg)	200	3	1.5	2	0	0	1	0	1.5	2.0	0
M.G.	7	1 (25 mg)	200	2	1.0	0	0	2	0	0	1.0	1.0	1
M.H.	17	1 (150 mg)	200	5	2.5	4	0	1	0	0	2.5	2.5	0
J.M.	16	1.5 (100 mg)	200	0	0	0	0	0	0	0	0	0	0
L.O.	13	2 (100 mg)	200	4	2.0	4	0	0	0	0	2.0	2.0	1
M.G.	42	0.5 (100 mg)	200	6	3.0	2	0	3	1	0	3.0	3.5	4
A.V.	22	3 (125 mg)	200	9 [a]	4.5	5	0	2	4	0	5.5	7.5	1
D.S.	26	3 (125 mg)	200	12 [a]	6.0	6	0	4	6	0	8.0	11.0	0
A.D.	27	7 5 years 200 mg 2 years 125 mg	153	14 [a]	9.1	6	1	3	4	3	11.1	12.4	1
Control group of 20 persons	10	—	1900	26	1.4	19	0	5	3	0	1.4	1.6	5

[a] Significant increase ($P < 0.005$).

exposed to suspected mutagens in vivo seems to be a sufficiently sensitive method which can be used as an indicator of human exposure. In our opinion, this testing method is essential and should be used whenever possible.

Acknowledgement

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