GENETICO-TOXICOLOGICAL STUDIES OF DIOXIN-CONTAINING ECOTOXICANTS LONG-TERM CONSEQUENCES IN THE SOUTH VIETNAM. CYTOGENETIC PARAMETERS OF CULTURED PERIPHERAL BLOOD LYMPHOCYTES OF RURAL INHABITANTS

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Dioxin's specific biological activity is considered now as a hormone-like influence on the genome expression in target-cells that can bring to function coordination disturbances in organism different systems and to alterations of the normal adaptive and responsive reactions in reply to homeostasis changes and various exogenous effects [1]. The significance of such dysregulatory and disadaptogenic alterations for future generations is one of the most arguable questions. The direct evaluation of the genetic risk for humans is impossible because of rather recent exposure to this chemical. But the results of animal laboratory tests [2,3] and natural populations analysis [4] suppose the need for this problem to be solved. One can't apply the negative results of pure dioxin mutagenicity analysis [2] to an assessment of dioxin's secondary activity as well as to genotoxic potentials of its metabolites [5].

The appearance and the medical and biological manifestations of Agent Orange (AO) and dioxin-containing ecotoxic factor(s) (DEF) exposure consequences were registered [6] in rather numerous risk groups of the South Vietnamese population. The cytogenetic investigations using human whole blood cultures were conducted in order to demonstrate the probable shifts in cellular homeostasis as well as in chromosomal structure stability caused by past exposure with dioxin.

MATERIALS AND METHODS

Socio-demographically and medico-biologically homogeneous statistically categorized [7] groups of males (aged 31-50) with and without past potential exposure to AO were examined. The control samples (n=10) were obtained from the inhabitants of Chanh My and Tuan An (TA) villages and the experimental seria was from the AO-exposed groups of Binh My (BM) village inhabitants (n=33). The AO-exposed were divided into two subgroups - according to direct (BMD) and indirect (BMI) contacts with AO. Whole blood cultures were started in RPMI 1640 with 15% calf serum and PHA (Difco) and lymphocytes were harvested 72 h and 96 h after the culture initiation. Bromodeoxyuridine (BDU, 10 mcg/ml) was present for 72 h or for the last 30 h of incubation, correspondingly. Cell cycle kinetics, chromosome aberrations, sister chromatid

exchanges (SCE), polyploid and endoreduplicated cells were analyzed on the same slides prepared by routine procedure and differentially stained by FPG technique [8].
A special index, or the rate of environmentally determined SCE (eSCE, %) was introduced [9] to distinguish the rates of genetically and environmentally determined SCE:

eSCE, % = (eSCE / sSCE) x 100% = (sSCE - gSCE) / sSCE x 100% , where

gSCE - is the SCE per cell frequency registered on the 96-th h and reflecting mostly the influence of genetic factors; sSCE - is the SCE per cell frequency registered on the 72-nd h and reflecting the "spontaneous" or "general" SCE level (genetically plus environmentally determined SCE); eSCE - difference between these SCE values, reflecting mostly the exogenous (environmental) factors influence [10].
Separate evaluations of the cells with high SCE frequencies (HFC) were implied. The values exceeding 10 SCE per cell were considered as high for the cultures registrating the sSCE, the values exceeding 7 SCE/cell were considered as high for cultures harvested on the 96-th hour. Whole blood cultures of several patients were loaded with mitomycin C (0.1 mcg/ml) and harvested on the 72-nd h.
Statistical analysis was performed using Student's t-factor comparison and ANOVA tests (Statgraphics v.3.0).

RESULTS AND DISCUSSION

The main results are presented in Tables 1-3. The cell cycle kinetics assayed by proliferative indices (PI) [11] have shown only a small decrease in the rate of cell proliferation in the cultures of BMD group patients. There were practically no cells with aberrations in the controls (only 1 cell with chromatid break per almost 2000 analyzed) and in the joint BM group 25% of patients bear the aberrant cells (1-2 cells per personal count): that were 29.4% in BMI group and only 6.3% in BMD group. The level does not exceed that one in normal populations, but the observed difference in distribution is rather notable. Almost the same picture was seen when the numbers of polyploid (4 n) and endoreduplicated cells have been analyzed (Table 1). It should be stressed that the endoreduplicated cells were seen much more frequently in the group of patients with the direct contact with AO. This may be considered as the first indication of cytogenetic changes probably leading to more serious problems (such as abnormal cell proliferation and carcinogenicity promotion).
The gSCE level was almost the same for all 3 groups under the study (Table 2) giving evidence for the similarity of this cytogenetic endpoint manifestation in cultured lymphocytes. The principle difference between the exposure risk groups can be seen by the eSCE values mainly characterizing the cell reaction on the environmental factors influence. The patients from the sprayed village had considerably higher eSCE values - in the majority of BM inhabitants the exceedence of 2.0 SCE/cell was observed - that is the highest value for those living in the intact region. To find out the influence of AO exposure in the past and current influence of dioxin-containing ecotoxic factor (DEF) the eSCE levels in BMD and BMI groups were compared.

Average eSCE values for the group BMD appeared to be almost twice lower then in the group BMI (1.74 ± 0.27 and 3.11 ± 0.28 SCE/cell, $p<0.01$). All the cases that determined the principle difference between the groups from the sprayed and the intact areas were the cases from the BMI group. The eSCE rates were much more pronounced in the group of patients exposed to

DEF ($p<0.01$). That's why we can state the strong influence of this complex environmental factor on the population living in the sprayed area.

Table 1. Cytogenetic characteristics of peripheral blood lymphocyte cultures obtained from persons of different Exposure Risk Groups, Song Be Province (South Vietnam)

Indices	TA	BMI	BMD
(Average ± S.E.M.)	(n=10)	(n=17)	(n=16)
PI (proliferative index), 72 h	1.82 ± 0.08	1.81 ± 0.05	1.63 ± 0.05*
PI , 96 h	1.56 ± 0.05	1.43 ± 0.04	1.47 ± 0.04
Aberrated cells frequency	1 per 2000	2-4 per 2000	
Patients with:			
chromosome aberrations, %	< 10	29.4	6.3
polyploid (4 n) cells, %	30	52.9	50.0
endoreduplicated cells, %	0	11.8	18.8

TA - Tuan An village inhabitants (control region); BM - Binh My village inhabitants with Agent Orange direct (BMD) and indirect (BMI) contacts; * - p = 0.04.

Table 2. Sister chromatid exchange (SCE) frequencies in peripheral blood lymphocytes of persons from different Exposure Risk Groups, Song Be Province (South Vietnam)

Indices	TA	BMI	BMD	p^+
(Average ± S.E.M.)	(n=10)	(n=17)	(n=16)	
sSCE, 72 h	5.95 ± 0.37	7.42 ± 0.37[a]	6.23 ± 0.30*	0.016
gSCE, 96 h	4.69 ± 0.31	4.30 ± 0.25	4.50 ± 0.16	0.550
eSCE (SCE/cell)	1.26 ± 0.20	3.11 ± 0.28[b]	1.74 ± 0.27**	0.0001
eSCE Rate, %	20.81 ± 3.06	41.45 ± 2.37[b]	26.78 ± 2.78**	0.0000
HFC (cells/count), 72 h	2.60 ± 0.94	6.94 ± 1.20[b]	2.81 ± 0.73**	0.0052
HFC (%), 72 h	6.50 ± 2.36	17.35 ± 3.01[b]	7.13 ± 1.83**	0.0053
HFC (cells/count), 96 h	3.80 ± 1.14	2.71 ± 0.78	3.44 ± 0.70	0.6540
HFC (%), 96 h	9.65 ± 2.82	6.76 ± 1.95	8.59 ± 1.75	0.6296

TA - Control village inhabitants, BM - Binh My village inhabitants with Agent Orange direct contact (BMD) and indirect contact (BMI); eSCE - SCE/cell difference between two cultures harvested on the 72-nd and 96-th h, reflecting environmental factor(s) influence; eSCE Rate - difference increase, %; HFC - cells with high SCE numbers;
+ - One-way ANOVA, significance level;
* - $p<0.05$, when compared with BMI level; ** - $p<0.01$, when compared with BMI level;
a - $p<0.05$, when compared with TA level; b - $p<0.01$, when compared with TA level.

Multifactor analysis and stratification of injuring factors according to pesticides and smoking habits showed no statistically significant difference between these 3 groups ($p=0.65$). Statistically significant association ($p=0.04$) was registered only for eSCE rate in smoking and exposed to DEF pesticides' applicators in BMI group. Only these persons in this group had endoreduplicated

cells in their cultures. However, the tendency of the SCE levels' elevation in those smoking was found in each of the studied risk subgroups (Table 3).

Table 3. Additive risk factors influence on the cytogenetic parameters in cultured peripheral blood lymphocytes of persons from different Exposure Risk Groups (ERG) (South Vietnam, Song Be province) [Average ± SEM].

ADDITIVE FACTORS		EXPOSURE RISK GROUPS	sSCE	eSCE	eSCE Rate	HFC, 72 h	HFC, 72 h
			SCE/cell		%	N	%
S	P	Controls					
M	E	n=5	5.92±0.82	1.27±0.27	21.06±3.52	2.6±1.47	6.5±3.68
O	S						
K	T	BMI					
I	I	n=7	7.77±0.73	3.64±0.55	45.62±3.54*	9.29±2.39	23.21±5.97
N	C						
G	I	BMD					
	D	n=8	6.65±0.73	1.99±0.49	28.08±4.66	4.13±1.26	10.31±3.15
	E						
	S						
		BMD					
NO	NO	n=7	5.89±0.36	1.49±0.27	24.87±3.73	1.71±0.52	7.14±2.46

BMI - Binh My village inhabitants with Agent Orange indirect contacts;

BMD - Binh My village inhabitants with Agent Orange direct contacts.

The registration of the cells with high SCE frequencies (HFC) - showed the same picture for the HFC distribution among these exposure risk groups (Table 2) - the high significance ($p<0.01$) of increase in the numbers of the HFC in cultures from the BMI group persons. The average percentage of the HFC in this group was 17.35%, in the BMD group - 7.13%, and in the control group - 6.50%.

The greater cytogenetic alterations observed in the group BMI can be considered as the manifestation of dioxin "paradoxical" effects or as a realization of adaptive process mechanisms in those with the direct exposure. Their somatic cell's defense against the consecutive TCDD small quantities' intake should have been provided on the level of enhanced reparation, longer cell cycles, and quick elimination of aberrated cells. The lower limits of cell reserve potentials (CRP) in persons with the direct AO contacts should bring to destruction of any cell or molecular structure with the exceedence of primary lesions [9].

The hidden changes in cells' homeostasis may be revealed only after the influence of other biologically active substances as it has been shown during the preliminary study of lymphocyte

cultures loaded with mitomycin C (MM) [9a]. The highest increase of the MM induced SCE (almost 5-times over the gSCE) was observed in the cells of the unsmoking person exposed to DEF. At the same time the minimal SCE relative frequency elevation (2.8-times) was registered in the cells of smoking person with the direct contact with AO in the past. Several years ago M.Lim et al. [3] mentioned the reduction of the induced by MM SCE level in lymphocytes of macaca-rhesus in the presence of TCDD activity in comparison to those in the intact animals. The susceptibility to xenobiotics is associated with the activity of genomic xenobiotics responsive elements (XRE) [12]. TCDD may play a significant role as a competitor for the DNA sequences that are functionally involved in the AhR-XRE formation, shifting the cells and organism normal response to exogenous factors influences. TCDD's antagonistic activity was registered for naphthoflavone, for ellipticine [13,14], as well as SCE induction suppression by naphthoflavone [15]. The AO exposure in the past may prevent an additional xenobiotics to interfere into the inducibility mechanisms. The results of cultured cells loading with mutagen may be of particular interest because MM is the direct acting mutagen and needs no activation through the monooxygenases' system. The mechanisms associated with the CRP exhaustion may be involved also. This and several other problems are discussed in the following reports also presented to the 14-th Symposium [16, etc.].

The additive effects of AO and other ecotoxicants producing the dioxin containing ecotoxic factor(s) can considerably alter the protective mechanisms of the cell and the whole organism. First of all they will affect the adaptive potentialities, the ability to survive the molecular lesions induced by the exogenous factors. The structures providing normal heredity realization can be affected as the result and this may bring to pathology risk increase in the population.

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