METHOD FOR REVEALING AND CHARACTERIZING THE CYTOGENETICAL COMPONENT OF THE LONG TERM MEDICAL CONSEQUENCES OF THE DIOXIN CONTAINING CHEMICALS APPLICATION

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The disadaptogenic activity of chemical substances like 2,3,7,8-tetrachlorodibenzo-p-dioxin ("Dioxin") was mentioned for the first time in 1992 [1]. Later it has been shown [2,3] that dioxins' hormone-like disregulatory and disadaptogenic qualities are manifested by the cascade of correlated pathogenic steps in the mechanism of sub clinical and clinical effects of the long-term medical consequences' syndrome formation. Separate sub clinical effects are combined now into the specific syndrome of the long term medical consequences of the dioxin containing chemicals' influence (DEFMC) [4]. In the contaminated regions the sanitary and remedial tasks are to be solved, so the specific methods for DEFMC sub clinical components revealing should be developed for this purpose [5]. These diagnostic methods could be based on the evaluation of the reserve potentials of organism systems different levels, including sub cellular. The changes in the chromosome structures are the most serious for an organism as these structures fulfill the function of inheritance information bearing. The special method is presented permitting to carry out the differential diagnostics of the chromosome structures' state in peripheral blood of persons from defined exposure risk groups. This method is based on registration of sister chromatid exchanges (SCE) in cultured lymphocytes induced by mitomycin C (MM) in vitro. The cellular disadaptive effects of dioxin containing ecotoxicological factor (DEF) thus may be studied by loading and provocative test, and by characterizing the reserve potentials of the cells, correspondingly.

MATERIALS AND METHODS

The differential analysis of genetically determined and caused by the environmental factors' influence SCE in human peripheral blood lymphocytes permits to reveal the genotoxic activity of the exogenous factors [6] as well as individual characteristics of the response to such influence [7]. The following parameters of the human chromosome structures were analyzed: SCE spontaneous level (sSCE) that combines the 2 components - genetically determined SCE (gSCE) and SCE caused by the exogenous, mostly environmental, factors influence (eSCE). The level of induced SCE (iSCE) registered after lymphocyte culture loading with MM was examined especially.

Two groups of South Vietnam inhabitants were examined with similar specificity of local conditions but principal differences according to exposure with Agent Orange (AO) and/or DEF [4]. The 1st group was selected among the inhabitants of control region - without any possible contacts with dioxin (N=10); the 2nd group was selected among persons with AO direct contacts during the war-time and those who had been living on the sprayed territory (N=33). The control and sprayed regions had obvious differences according to dioxin residuals content in soil, sediments, as well as in tissues of fish, mammals and humans [2].

Whole blood cultures were harvested on the 72nd (for sSCE) and 96th (gSCE) h of incubation in RPMI 1640 with 15% calf serum and PHA (Difco). Bromodeoxyuridine (BDU, 10 mcg/ml) was present for 72 h or for the last 30 h of incubation, correspondingly. SCEs were analyzed on the slides prepared by routine procedure and differentially stained by FPG technique. Forty metaphase cells were examined for every culture and every fixation point.

The levels of SCEs and the rate of eSCE were analyzed according to the formula: eSCE, % = (eSCE/sSCE)x100% = (sSCE-gSCE)/sSCEx100%, where eSCE - difference between spontaneous and genetically determined SCE values, reflecting mostly the environmental factors influence [7].

Separate cultures were loaded with mitomycin C (0.1 mcg/ml) at the start point and harvested on the 96th h for the analysis of an induced SCE level (iSCE).

Statistical analysis was performed using Student's t-factor comparison and ANOVA tests (Statgraphics v.3.0).

RESULTS AND DISCUSSION

The application of special loading tests in sub clinical DEFMC effects examination on different levels (molecular, cellular, system, organism and functional) [5] allowed to find out the development in the sprayed region of individual specific states with predisposition to AHH hyperinducibility, with vit A homeostasis system alterations (reflecting the contradictions appeared between the vit A consumption and catabolism), with altered adaptation of visual receptors, with functional abnormality in oxygenating system, with functional decrease in working capacity (physical and mental). The immunodeficiency in these people correlated with the chromosome structures' destabilization in their lymphocytes.

The homeostasis disturbances and structure-functional destabilization in residents of the sprayed region are determined by the decrease in the reserve potentials of all systems and levels as the result of DEF disregulatory activity [5]. On the cellular level we registered the paradoxical changes in the level of SCE induced by MM, i.e., in iSCE. Thus the cultures loading with this mutagen permitted to reveal the decrease in the rate of eSCE (induced on the background of those already existent) in the cells of persons living in the contaminated region (Table 1).

Table 1. Sister chromatid exchanges (SCE) levels in peripheral blood lymphocytes of persons from the different exposure risk groups

Parameters	Without loading		With mitomycin C loading	
	Exposure risk groups			
	1	2	1	2
sSCE	5,95±0,37	6,84±0,26*	23,83±0,82	16,17±0,48**
gSCE	4,69±0,31	4,40±0,15	4,16±0,21	3,86±0,20
eSCE	1,26±0,20	2,45±0,23**	19,12±0,71	12,06±0,60**
Rate of eSCE, %	20,81±3,06	34,34±2,21*	80,23±2,11	74,58±1,36

* - $p<0,05$; ** - $p<0,01$.

The cytogenetic parameters' significance increase can be seen in the following order: gSCE<sSCE<eSCE<iSCE. This dynamics may be associated with an alteration in DNA repair systems homeostasis after functional loading on the cultured somatic cells. The routine harvesting time for SCE analysis (72 h) may reflect the complex potentials for cellular vitality provision (including reparation, replication, membranes' permeability, receptors' activity, and AhR as well; any structural unit reactivity; and effects of exo- and endogenous factors on the intracellular processes). The sSCE level may serve as the marker of cellular reserve potentials (CRP) for self reproduction. The gSCE level is analyzed in cells entering the replicative synthesis in the presence of BDU after the first division passed in the medium without this marker required for chromatid exchanges manifestation. The cells have spent up to that time the part of their CRP for reparation of primary lesions formed during the 1st replication cycle. These lesions may be recognized and repaired by the cell only after the mitotic stimulation and leaving the Go phase. The synthetic phase undoubtedly provides the restoration of CRP. However, the proliferation of untransformed cells (as lymphocytes) in culture can't pass without the CRP exhaustion. The gSCE level is defined by the limits provided by the CRP exhaustion. Practically the same level of SCE had been registered in the following divisions [6].

The eSCE levels characterize not only the level of chromatid reorganizations induced by environmental factors but the activity of systems for cell recovery as well. The experimental conditions practically permitted to associate the eSCE level with the CRP spending for lesions repair and homeostasis provision and to consider this parameter as the marker for CRP expenditure. This marker's significance is enhanced while homeostatic systems loading by mutagenic induction. The additive DNA lesions induced by MM require extraordinary CRP spending that exceeds the possible limits in the cells of people from the contaminated region. Thus, the level of the induced SCE in their cells was much lower ($p<0.01$).

It is very difficult to suppose MM uninterference into the fate of lesions leading to gSCE and its effect only on the surplus (exceed) exchanges - manifested as the eSCE. Nevertheless However, if the hypothesis on an additive activity of MM could be admitted, the level of the SCE induced by this mutagenic substance would be determined approximately by the difference of the eSCE rate in the cultures loaded with MM and those without loading. In our experiments the rates of these MM-induced exchanges were 60% for the control region's cultures and about 40% for the

cultures obtained from the sprayed region. Several years ago M.Lim et al. [8] also mentioned the reduction of the sister chromatid exchanges level induced by MM in vivo in lymphocytes of macaca-rhesus in the presence of TCDD activity in comparison to those in the intact animals.
Thus, the use of this loading test allows to characterize the CRP and to obtain the spectrum of the sub clinical alterations in the somatic cells cytogenetic parameters. The conditions are provided for DEFMC diagnosis according to the development of medically significant alterations on the background of already existing sub clinical changes in chromatid's structures that have been induced by the environmental factor(s).

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