HIGH OXIDATIVE COMPONENT IN THE MECHANISMS OF PCDD/F ACTION CAN LEAD TO ITS SEEMING NON-MUTAGENICITY FOR HUMANS

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Introduction

The most striking feature among known molecular mechanisms of PCDD/F action appears to be Ah receptor - dependent induction of microsomal cytochromes P-450, which has good correlation with toxicity of different congeners for laboratory animals. As from pioneer works of S. J. Stohs's group (Creighton University) there are also sufficient quantity of experimental data demonstrated by different methods that oxidative stress occurs in various tissues of PCDD/F-treated animals [1 - 5]. Such classic signs of oxidative stress as superoxide anion production and lipid peroxidation were revealed usually in parallel with single strand breaks of DNA from the same tissues of treated mice or rats. Being theoretically extrapolated on human population, these data can be simple and reasonable explanation of well documented increased cancer risk among exposed by PCDD/F human cohorts. Nevertheless cytogenetic data received during biomonitoring of occupationally or environmentally exposed human persons are very contradictory [6 - 9] and leave in the whole impression that PCDD/F can't be refer to the substances which are obvious mutagenic for human population. As to the other side of the problem, possible involving of oxidative stress in the mechanisms of PCDD/F's action onto exposed human persons represent now annoying gap in general knowledge about harmful effects of these persistent organic pollutants. It was shown only by M.Ernst et al. [10] that neutrophils from TCDD-exposed donors had significantly higher ROS-dependent chemiluminescence in answer to different phagocytic stimuli.
During recent pilot medico-genetical observation of russian town Chapaevsk residents we have received a possibility to evaluate possible signs of PCDD/F's-connected oxidative stress in exposed human organisms and to compare obtained free radical and cytogenetic parameters. The results were partly discussed previously during 1st All-Russian Conference of Toxicologists [11].

Material and methods

Samples of venous blood and mixed saliva were transported on ice to Moscow, where cells were sedimented and withdrawn. Supernatants were stored at – 18°C.
Luminol-dependent chemiluminescence (CL) was measured in a solution which contained 0.14 M Na-phosphate buffer (pH 7.4), 50 mkM luminol, 10 mkl/ml of blood plasma or 50 mkl/ml of saliva; the reaction was initiated by addition of hydrogen peroxide.
Spontaneous peroxidative hemolysis (3 h 37°C) was determined as a measure of cell membranes sensitivity to oxidative damage.
Levels of chromosomal aberrations (CA) in whole blood cultures of observed persons were scored in Medico-Genetical Center, Moscow by V.Platonova and L.Katosova.

For 14 persons from total 45 direct chemical analysis of PCDD and PCDF blood burdens was carried out by E. Brodsky and N. Kluev (A. N. Severtsev's Research Institute of Ecology and Evolution, Moscow).

Results and discussion

Oxidative stress Tight positive correlation (up to r 0.75, $p \leq 0.002$) was revealed between induced by H_2O_2 blood plasma luminol-dependent CL and different PCDD/PCDF congeners content in plasma lipids; this result was in full accordance with corresponding data shown previously for TCDD treated animals.

Even slightly more tight but *negative* correlation was obtained for saliva CL and the toxins burdens. Such unexpected effect is likely a consequence of low lipid but high peroxidase content in saliva comparing with blood plasma as for a long time studied object of CL analysis. Salivary peroxidase is mainly product of local neutrophils secretion, so observed expressive decrease of saliva CL among the persons with high PCDD/PCDF burdens can be a sign of phagocytes functional depression in oral cavity, which then must be associated with increased sensitivity of inhabitants in polluted areas to various respiratory infections. Two parallel lines on both scatterplots (Fig.1) represent apparently two human subpopulations with different balance of pro/antioxidative reactions; analogous effect we have described previously for erythrocytes oxidative stability in human population [12].

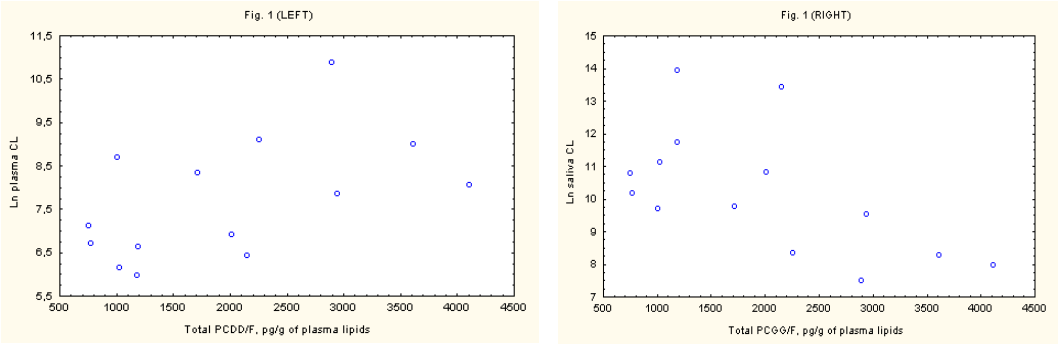

Fig.1 XY scatterplots for 14 observed persons with directly determined PCDD/F blood content: Y axis: blood plasma CL (left graph) and saliva CL (right graph) as Euler logarythms

Coefficient of reciprocal correlation for the obtained values of blood plasma and saliva CL was equal to - 0,850 ($p < 0,00012$) among observed in the same day 14 persons with known PCDD/F's content and –0,621 ($p < 0,0002$) for those 32 pairs of samples which were collected during sufficiently long period between january and march 1998, but had identical regime of transportation (one day at 2 - 4°C). Probes from remaining 13 persons were transported to Moscow within 2 days; so Pearson's correlation between plasma and saliva CL for all 45 observed persons had considerably diminished coefficient (-0,398 $p < 0,007$).

Peroxidative hemolysis values for the same blood samples had no direct correlation with the toxins burdens; there was a tendency to even slightly elevated (probably adaptive in answer to increasing of free radical processes in plasma) oxidative stability of washed erythrocytes among the observed persons having more significant occupational input of dioxins.

Relationship between free radical and cytogenetic parameters Standard statistical analysis of cytogenetical data have revealed lack of direct correlation between CA and PCDD/PCDF blood burdens; in the row "relative control – colony near plant – herbicide plant" all CA types have increased but unsignificantly except for significant increasing of "fresh" chromosomal exchanges (Zhurkov et al., 1999, in press).).

Nevertheless sufficiently close relations can be revealed between cytogenetic damage and CL intensity. Total CA levels have increased proportionally to CL intensity for moderately exposed persons living near plant, while sharp fall of the curve was observed for highly exposed plant workers (Fig.2; variable "transday" corrects data scattering connected with different time of samples tranportation; for subcohort of minimally exposed relative control donors, which isn't shown at the graph, curve shape is similar to the right one, but with much less inclination).

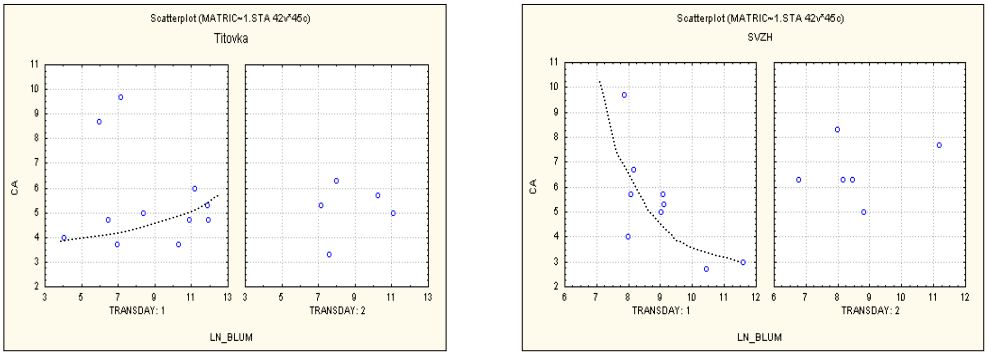

Fig. 2 XY Scatterplots for moderately (left) and highly (right) exposed by PCDD/F subcohorts of Chapaevsk residents (subcohort of relative controls isn't shown, see text)
X axis – Euler logarythms of blood plasma CL intensity;
Y axis - CA level in blood lymphocytes of the same persons
Transday: time period of samples transportation in Moscow (1 or 2 days)

Shape of CA distribution for the subcohort of plant workers looked like mirror variant of Poisson-like "random events" distribution for relative controls with additive group of outliers having highest CL signals on its left flank - the fact which can be also treated as an evidence that in this most exposed subcohort DNA damage appoints to its maximum and begins to drop Such results can be easily explained if we shall postulated that generalized oxidative stress, which we have registered by blood plasma CL increasing, is a single reason for DNA breakage in lymphocytes of PCDD/F exposed persons. It is known that DNA cleavage by ROS goes mainly via sugar moiety disruption, so process of DNA oxidative damage must always go in parallel with damage of other sugar-contained cell target - outer membrane polysacharids. Sufficiently significant alteration of

specific polysacharid pattern is a sign by which oxidatively damaged cells can be recognized and eliminated by macrophagues, and this delayed macrophagal reaction is a reason of transient CA rise among moderately exposed human persons with subsequent quick decreasing of mutagenic effect among the most highly exposed persons. Since all investigators strive for including in observation the most exposed persons, which have already falling signs of cytogenetic damage, we have contradiction between appearing unmutagenicity (or very weak mutagenicity) of PCDD/F for human population and increased cancer risk in polluted areas. Paradoxically but statistically significant differences between control and exposed donors in this case must be evaluated namely when cytogenetical observation is carried out in the area with not very high levels of pollution. It is interesting that as a result of very large observation [13] mean level of sister chromatide exchanges in blood lymphocytes of vietnameses in moderately polluted by Agent Orange villages was significantly increased but the same value for the persons from highly polluted villages have decrease almost to control values. Analogous results were published also for Chernobyl liquidators [14] having DNA damage of generally accepted oxidative origin.

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