

Confounder factors influence on two types of human cells on the territory sprayed with Agent Orange

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Abstract

The accumulation of nuclear damages in cells of different origin (epitheliocytes, erythrocytes, etc.) is defined in human samples from village sprayed in the past with Agent Orange (AO). The frequencies of nuclei's aberrations produced in human cells were analyzed according to the possible influence of confounding factors. No influence of contacts with pesticides in general was observed in the sprayed with AO region. Individual contacts with Bazudin or 2,4-D on the sprayed territory induced the higher frequencies of damaged nuclei in erythrocytes and in buccal epitheliocytes. The associations were found also for food products and micronucleated cells' frequencies. The higher correlation coefficients were registered for rice-dishes and eggs' consumption by persons living in the contaminated region.

So, the higher rate of appearance of nuclei's damages, micronucleated and binucleated cells, cells in apoptosis and perforated (punched, necrotic) cells testify to a decrease in self-protective mechanisms in cells of individuals exhibiting contacts with dioxin-containing ecotoxicological factor (DEF). Thus, an increase in carcinogenic processes may be provided if induced by the contacts with common environmental factors - smoking, domestic chemicals, pesticides, drugs, etc.

Introduction

Abnormalities of nuclear material and their persistence in different tissues^{1,2)} of persons living on the contaminated with AO area testify to the presence of primary lesions in stem cells. These lesions could be expressed in differentiated cells undergoing strong influence of additive hazardous factors.

The peculiarities of environmental factors influence on distribution of different micronucleus' morphological types were found in South Vietnam inhabitants. It was shown during the examination of epitheliocytes from females' buccal mucosa and of peripheral blood erythrocytes^{3,4)}. The higher frequency of nuclear structures' instability was discovered in females exposed to AO. However the influence of environmental factors on contaminated territory is not limited to influence only. Many local effects may contribute to DEF effects. The examined females are in contact with agriculture pesticides and other adverse factors (e.g. drugs). They may be exposed also to the genotoxic substances probably present in food products. Special study was designed to verify the observation on total DEF influence and to find out the influence of separate confounder factors.

Object

One hundred persons were examined in two villages from Song Be province (South Vietnam). Epidemiological data and smears of buccal mucosa and peripheral blood were collected by the specially trained personnel of the Song Be province Family Planning Center. Investigation of microscopic slides (stained with Giemsa) was carried out in the Tropical Centre according to earlier defined nucleus structures morphological types³⁾.

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Fifty thousand of desquamous cells of buccal mucosa and 100000 peripheral blood erythrocytes were analyzed in blind from 50 women (average age $38,24 \pm 8,16$) of Binh My village with a history of AO spraying. In the control village Tan An (40 Km far away) matched contingent of 50 persons was analyzed ($38,46 \pm 8,27$).

Statistical analysis was carried out with use of the software packages "Statistica, v.4.3" and "Statgraphics, v.3".

Results and Discussion

Tables 1 and 2 summarize the significance of alterations registered in the analysis of cytogenetical and morphological parameters of buccal mucosa epitheliocytes and peripheral blood erythrocytes. The frequencies of the cytogenetic parameters established by the analysis support the data of previous publication³⁾ and agree with those reported by⁵⁾. That concerns the altered distribution of nucleus' defects in two Exposure Risk Groups matched by most parameters but exposure to AO.

A separate analysis of contacts with particular pesticides demonstrated the following situation in the younger group (30-40). Positive correlation was real between very small-sized micronuclei (MN) in erythrocytes and contact with Bazudin ($p=0.012$) or 2,4-D ($p=0.045$). The same tendency was shown for the mucous epitheliocytes with significant association of binucleated cells and contacts with Bazudin ($p=0.034$) and 2,4-D ($p=0.024$). Genotoxic potential of these chemicals was reported earlier⁶⁾.

Table 1. The significance of alterations in nuclear structures' parameters for buccal mucosa epitheliocytes of persons exhibiting different environmental factors exposure (South Vietnam)

Age group	p*			
	30-40		> 40	
Risk factors [#]	Total DEF -/+	Pesticides -/+	Total DEF -/+	Pesticides -/+
Cells analyzed	32000 /34000	20000 /14000	18000 /16000	9000 /7000
Cells with micronuclei:				
Type 1	0.012*	0.292	0.013*	0.055
Type 2	0.044*	0.527	0.011*	0.158
Type 3	0.694	0.201	0.038*	0.449
Type 4	0.380	0.826	0.001**	0.085
Sum of micronucleated cells	0.087	0.916	0.0000***	0.396
Binucleated cells	0.072	0.295	0.018*	0.094
Cells with fragmented chromatin	0.111	0.266	0.189	0.286
Sum of defected cells	0.006**	0.739	0.0000***	0.633

* - Mann-Whitney test

- Pesticides influence was analyzed for the inhabitants of the sprayed region only.

The higher revealing potential of the epithelial cells' analysis for the environmental factors general influence was mentioned in the report from this issue⁴⁾. At the same time, an independent comparison of the additional risk factors' effects only in sprayed region has shown absence of actual changes in mucous cells in the case of loading with pesticides. However, analysis of such loading with application of blood cells revealed significant changes among people of senior age group (table 2). The accumulation of damaged cells occurs owing to the increase of type 1 micronuclei, i.e. by formation of small-sized chromatin fragments.

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Table 2. The significance of alterations in nuclear structures' parameters for peripheral blood erythrocytes of persons exhibiting different environmental factors exposure (South Vietnam)

Age group	p*			
	30-40		> 40	
Risk factors [#]	Total DEF - /+	Pesticides -/+	Total DEF - /+	Pesticides -/+
Cells analyzed	66000 /66000	40000 /28000	34000 /32000	18000 /14000
Cells with micronuclei:				
Type 1	0.217	0.769	0.465	0.023*
Type 2	0.036*	0.595	0.008*	0.004**
Type 3	0.755	0.292	0.601	0.221
Type 4	0.448	0.601	0.969	0.264
Sum of micronucleated cells	0.418	0.912	0.149	0.004**

* - Mann-Whitney test

[#] - Pesticides influence was analyzed for the inhabitants of the sprayed region only

This fact corresponds to a general ageing tendency and decrease of reparative processes' efficacy in cells of elder persons⁷⁾. On the other hand, such tendencies are observed at younger (native) inhabitants of sprayed region⁸⁾ this time in mucous cells (see fig. 1 from ⁴⁾). Thus, once again the probability of stem cells' affection in the contaminated area is emphasised. The shift of natural ageing process to the younger age is obvious as well among the inhabitants of the sprayed village.

From our point of view, an incidence of such changes can be influenced by other risk factors as well. For example, genotoxic substances could be obtained with food products produced on the contaminated territory. The special analysis was launched to consider such question.

The comparison of main products consumed by the villagers is presented in Table 3. Only individuals without any contacts with pesticides, smoking, and any other harmful effects were selected for such evaluation. In the control region the appearance of MN in erythrocytes correlated ($p < 0.05$) with the amount of meat ($r = 0.49$) and fish ($r = 0.38$), as well as with eggs' number ($r = 0.40$ for the sum of MN-cells). In the sprayed region the significant correlation ($p < 0.05$) was shown for eggs ($r = 0.51$, type 1 MN and $r = 0.46$, type 4 MN) and oil quantity ($r = 0.66$ for the sum of MN-cells).

The correlation between food products and damages of nuclear structures was observed mainly for blood cells. This was practically absent for buccal cells. It is obvious, that most substances from food penetrate into an organism after metabolic fermentation, just by blood absorption. Thus, the genotoxic substances and their active metabolites may induce damages primarily in blood cells. In the control region the excess of meat consumption is evident. But it may produce an additional influence of genotoxic factors⁹⁾ as meat is consumed mainly as roasted on the open fire.

Table 3. The amount of main food products consumed every week by females in two villages from South Vietnam

Products per week	Tan An (N=32)		Binh My (N=20)		p
	Mean (S.E.M.)	Limits	Mean (S.E.M.)	Limits	
Meat, g	442.19 (55.90)	0-1200	270.00 (51.86)	0-1000	.040*
Fish, g	319.69 (42.70)	0-1000	242.50 (35.22)	50-800	0.211
Milk, l	221.25 (82.43)	0-2000	37.50 (25.10)	0-500	0.091
Eggs	1.72 (0.18)	1-6	1.30 (0.20)	1-3	0.135
Oil, ml	100.94 (9.65)	0-300	133.75 (27.93)	30-500	0.198
Rice, g	2540 (84.23)	2000-3500	2820 (150.72)	2000-4700	0.086

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At the same time, in sprayed region the correlations with food products are also observed, but absolutely for the other set of products. So, the correlations with the amount of consumed rice was registered for MN of the 1-st and 2-nd types, and also for the sum of micronucleated and apoptotic cells in mucosa ($\delta=0.0001$, $\delta=0.030$ and $\delta=0.032$, accordingly). Under our assumptions, in this case not just the amount of rice, as such, should be considered, but sauces and other dressings consumed with rice - the soy-been sauces and fishy sauces. They accompany any rice-dish as necessary food additive. Moreover, in these cases an organism meets already fermented product and absorption starts in oral cavity. Thus, the excess of rice-dishes supplied with sauces and vegetable oil may induce increased frequencies of MN-cells in buccal mucosa. This supposition can be testified by the positive correlation ($\delta=0.045$) of total number of micronucleated erythrocytes with the amount of vegetative oil consumed by the inhabitants of contaminated village.

An additional assay was started to evaluate the effect of the malaria disease on the cytogenetic parameters. No influence was shown in the control region, and in the younger age group in Binh My village. In the elder persons (> 40 , $N=6$, with no other effects) in the sprayed region the Spearman rank order correlation revealed the inducible effect of the malaria disease on the total sum of micronucleated epithelial cells ($r=0.82$, $p=0.04$).

Consequently, the dysadaptive effect of AO is confirmed. On the background of AO biological activity the probable increase in cellular injury is more obvious. This may be displayed by alterations of cellular (including nuclear) structures under influence of contacts with common factors (food products and their metabolites, professional hazards, drugs). These processes can be provoked by lowered ability of cells to restore the arising damages. That is evident considering inability to produce necessary plastic structures (proteins, enzymes, glycoproteins, etc.), as well as reduced energy capacity^{10,11}.

On the other hand, a green tea is certain and rather intensive protective factor for the South Vietnam's residents. From our point of view, the everyday use of plenty amounts of green tea not only promotes receiving of vitamins and mineral additives, but also endures protection for nuclear structures. Besides vitamins (well known antimutagens¹²), chlorophyll possesses such protective activity. That's why the frequencies of nuclei's damages, and the corresponding incidence of malignant processes in South Vietnam is not so high as could be supposed. The carcinogenicity may be masqueraded by the consumption of many natural products exhibiting adaptogenic and antimutagenic activities.

Conclusions

The stratification of confounder factors influences on the nuclear structures of females living in two South Vietnamese villages demonstrated the following. No general influence of contacts with pesticides was observed in the sprayed with AO region. Nevertheless the individual contacts with Bazudin or 2,4-D induced the higher frequencies of damaged nuclei both in erythrocytes and in mucous epitheliocytes of buccal cavity. The particular associations were revealed for dietary products and MN-cells' frequencies. The higher correlation coefficients were registered for rice-dishes and eggs' consumption in the contaminated region.

In the sprayed with AO region a decrease in self-protective mechanisms is obvious by the appearance of the nuclei's damages, micronucleated and binucleated cells, cells in apoptosis and perforated (punched, necrotic) cells. These may provide an increase in carcinogenic processes induced by the contacts with common environmental factors - smoking, domestic chemicals, pesticides, drugs, etc.

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