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HLA Typing and Chromosomal Aberrations Caused by Chemical Agents

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Abstract

The aim of this research was to perform HLA typing and determine the chromosomal aberrations in patients with chronic lead and pesticide (organophosphates and organochlorines) toxicity.

Materials and Methods: Cytogenetic studies were performed on 105 study participants. Eighty-five patients with chronic professional toxicity were divided into two groups. Group 1 consisted of 40 patients with chronic lead toxicity; Group 2 consisted of 65 patients with chronic pesticide toxicity. Cytogenetic effects were estimated using a metaphase plates technique. Cytostatic effect of studied chemicals was evaluated using PHA stimulation index. Distribution of HLA- antigens was studded in 30 patients with chronic viral hepatitis and 60 patients with work-related chronic liver disease.

Results: The frequency of chromosomal aberrations in peripheral blood lymphocytes was increased in persons with occupational exposure to pesticides. In 66.7% of workers exposed to lead, the response to PHA stimulation was very low. Our results showed that HLA- A2, A25, B16, B8, B22 antigens were determined statistically more frequently in patients with chronic liver toxicity; HLA-A2/B8, A2/B15, A25/B22 haplotypes increased the risk of liver toxicity.

Keywords: *chronic lead and pesticide toxicity; chromosomal aberrations; HLA antigens.*

Introduction

In life, a human is exposed to a large number of different chemicals that can be ingested in various ways. Due to the development of industry and transport, the population of large cities is strongly affected by heavy metals and other toxic-chemicals [1-3]. Among them the most common are lead, chromium, copper, cadmium, aromatic hydrocarbons, organophosphate, and organochlorine pesticides. The compounds of these substances have an adverse effect on the functional state of the nervous system, digestive organs, cells of the pancreas, and the mucous membrane of the small intestine [1,4,5].

Peripheral blood lymphocytes circulate throughout the body as carriers of many physiological functions. Irradiation, chemicals of an exogenous and endogenous nature, and viruses affect the genome, causing damage to it. The cells are subject to these three processes: slowdown of the cell cycle, DNA repair, and death [4]. High concentrations of lead compounds have mutagenic, gonadotropic and embryotropic actions [6]. It has been determined that chromosomal aberrations occur after exposure to chemicals *in vivo* [7]. Chromosomal aberrations have also been found during drug treatment. After patients with rheumatism received acetylsalicylic acid, the frequency of chromosomal aberrations was 2.5 times higher than before treatment. In cyclophosphamide-treated patients with malignant tumors, the proportion of aberrant metaphases increased from 3% to 37%. An examination of persons with potentially mutagenic factors in working conditions revealed an increase of 2–3 times in the frequency of chromosomal aberrations. Different approaches are used to study the genetic health of the population, including assessment of the frequency of chromosomal mutations in human somatic cells.

HLA and disease is one of the main sections of clinical immunogenetics that attracts the attention of many researchers. The unique features of the molecular organization of the HLA complex with an exceptionally high degree of genetic polymorphism allow extensive use of the HLA test in population/anthropological studies. Currently, the associations between the different HLA-antigens and a number of human diseases have been described, and in some of them, such associations are so obvious that often the identified antigen is regarded as a marker of the disease. At the same time, for liver

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toxicity this problem has not been studied fully. A detection of the marker HLA antigens will contribute to the early prediction and prevention of these chronic toxicities.

The aim of this research was to perform HLA typing and determine the chromosomal aberrations in patients with chronic lead and pesticide (organophosphates and organochlorines) toxicity.

Materials and Methods

The study included 30 patients with chronic viral hepatitis and 60 patients with work-related chronic liver disease. Written informed consent was obtained from each patient. We studied the distribution of HLA- antigens in all patients. HLA typing was performed in the tissue typing laboratory with a standard two-stage micro*lymphocytotoxicity test*.

Cytogenetic studies were performed on 105 study participants. Eighty-five patients with chronic professional toxicity were divided into two groups. Group 1 consisted of 40 patients with chronic lead toxicity; Group 2 consisted of 65 patients with chronic pesticide toxicity. Work experience of patients was more than 10 years. The occupation of Group I participants was linotype operator (direct contact with lead) and of Group II the occupations were agronomist and entomologist. The control group included 30 healthy age- and sex-matched individuals.

Venous blood was collected and placed into vials with 199 culture medium (6 ml) containing a phytohemagglutinin (PHA) and bovine serum. Cultivation was conducted for 72 hours in an incubator at 37° C. Two hours before the end of cultivation, colchicine was added to the vials with the cell mixture to stop mitosis. After cell fixation, samples of metaphase plates were prepared. Romanowsky-Giemsa staining was carried out. Chromosome analysis was performed with a Leica microscope, eyepiece 10, the lens 100. The proliferation stimulation index (SI) (ie, the ratio of PHA-stimulated peripheral blood lymphocytes proliferation/ spontaneous peripheral blood lymphocytes proliferation) was calculated.

Statistical analysis was performed using a statistical software package, Statistica 6.0. Analysis of the distribution of values obtained was performed using the Kolmogorov-Smirnov test. For data with normal distribution, inter-group comparisons were performed using Student's t-test and F-test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

In Group 1 (patients with chronic lead toxicity), the frequency of chromosomal aberrations was increased, compared to the control group. In Group 2 (patients with chronic pesticide toxicity), we found a tendency to induction of chromosomal aberrations (Table1).

The structure of chemical mutagens has a great influence on the severity of disturbance in chromosomal structure. The role of these mutagens in the induction of chromosomal aberrations is well studied. Substances containing more than one active group (3 or more) often do not lead to an increase in chromosomal aberrations, but a monofunctional agent such as methyl methanesulfonate or ethyl methanesulfonate may possess pronounced mutagenic ability. The cytotoxic effect of each ingredient depends upon the reactivity of the active groups, and the ability to penetrate the cell and to interact with components of the chromosome. In our study, the unequal mutagenic effect of the studied factors apparently was also associated with their chemical nature.

Mechanisms of induction of chromosomal aberrations are extremely diverse. The action of chemicals can be carried out not only by direct interaction with the structural components of the chromosomes, but also through their effect on cellular metabolism.

Table 1.

The frequency of chromosomal aberrations in patients with chronic lead and pesticide toxicity

Groups	Number of metaphases	Number of aberrant metaphases	Number of aberrations	SI
Control group	500	12	2.4 ± 0.11	0.09
Group 1	50	2	4.0 ± 0.88*	0.01
Group 2	150	4	2.7 ± 0.22	0.03

**-P<0.05 vs control group*

In our study, the frequency of chromosomal aberrations in peripheral blood lymphocytes was increased in persons with occupational exposure to pesticides. Among the identified aberrations, divisions in chromosomes of A(1st–3rd pairs), C(6th–12th pairs), D(13th–15th pairs), and E(16th–18th pairs) groups, fragments of different sizes, dicentric chromosomes, and fragments with displacement were the most frequent. Investigation of the distribution of cytogenetic damage in cells under the action of chemical mutagens might help in understanding the nature of the interaction of the damaging agent with cell components.

Different agents may have mutagenic and cytotoxic properties. Cytostatic effects of the substance appear in the delayed stimulation of mitosis in cells cultured with PHA, which can affect the results of cytogenetic analysis. In 66.7% of workers exposed to lead, the response to PHA stimulation was very low. Activation of lymphocytes with PHA that is accompanied by their blast transformation and proliferation is seen as an important indicator of the functional state of the immune system, in particular, T cells; therefore, the low response of lymphocytes to PHA stimulation in individuals may indicate an immunodeficiency state. The presence of the induced chromosomal aberrations indicates the mutagenic effect of production factors.

According to the obtained results, in people exposed to lead and pesticides a mutation process was detected, which can be a cause of spontaneous abortions, congenital malformations, hereditary diseases, and other pathologies.

In this connection, prediction of mutagenicity of production factors is a critical need. Prediction of the induced mutagenesis allows us to evaluate the intensity of mutation in somatic cells, which leads to carcinogenesis, and in germ cells, which leads to accumulation of the genetic load and hereditary diseases. Evaluation of the genetic effects of chemicals based on experimental genetic and sanitary data can provide primary "inventory" or identification of mutagens and establish the quantitative laws of the mutation process. With these data we can determine the value of the "mutagenic load" under the influence of the various substances, their interaction, and combination with other factors.

Our results showed that HLA- A2, A25, B16, B8, B22 antigens were determined statistically more frequently in patients with chronic liver toxicity. The presence of these antigens in the HLA phenotype of individuals increases their risk of toxic hepatitis. We also studied HLA haplotypes and found that HLA-A2/B8, A2/B15, A25/B22 haplotypes increased the risk of liver toxicity. The findings suggest the presence of HLA-associated genetic control in the transmission of susceptibility to toxic liver disease.

In conclusion, clinical and genetic studies should be widely used to determine individual risk of occupational disease in order to develop the best methods of prevention and treatment of diseases, management of the employment of persons with a specific phenotype and genotype.

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