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Genetics

REACTIVATION OF HETEROCHROMATIN INDUCED BY SODIUM HYDROPHOSPHATE AT THE OLD AGE

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(Received July 28, 2003)

Abstract

It has been stated, that progressively developing process of heterochromatinization (condensation of eu- and heterochromatic chromosome regions accompanied by genes inactivation) occurs at aging. In this connection, we consider it expedient to determine whether the system of chromatin domains undergoes changes at aging when exposed by the sodium hydrophosphate. Influence of sodium hydrophosphate was studied on the level of chromatin condensation in PHA-stimulated of cultured lymphocyte derived from persons at the age of 70 - 80. The data obtained indicate that at aging sodium hydrophosphate induces: 1. activation of synthesic processes, as a result of reactivation of ribosomal genes via deheterochromatinization of nucleolus organizer regions; 2. decondensation of pericentromeric structural heterochromatin (C-bands of chromosomes 1, 9 and 16); 3. Reactivation of facultative heterochromatin, the genes repressed due to condensation of euchromatic regions.

Key words: aging, aberration, heterochromatin (structural, facultative), heterochromatinization, N0R, SCE, C-bands.

Introduction

Aging is defined as a manifestation of complex changes in genetic processes that lead to the gradual functional disorders giving rise to senile diseases resulting in inevitable death of an organism. Hence it appears necessary to develop new chemical preparations for slowing down "the biological clock" and preventing senile pathologies. A special interest is paid to sodium hydrophosphate, which stimulates ATP synthesis and extension of cells lifespan. As a result of sodium hydrophosphate activities some metabolic changes regulated through the genes in chromatin domains occur [4,5].

It is well established that chromatin is composed of distinct functional domains. Heterochromatin includes constitutive heterochromatin almost entirely composed of noncoding sequences of satellite DNA and facultative heterochromatin (condensed euchromatic regions), that mainly consists of "closed" transcribable genes [1].

In support of this suggestion the established data testifying that presence of only "active genes" is not enough for transcription, but existence of "active chromatin" is required as well [2]. It has been suggested, that progressive heterochromatinization accompanied by genes inactivation occur at aging [6].

In this view, we considered it expedient to determine whether the system of chromatin domains in cultured lymphocyte from old persons undergoes changes when exposed by the sodium hydrophosphate. In particular, our aim was to study the variability of the levels of chromatin condensation: nucleolus organizer regions; structural heterochromatin; and facultative heterochromatin.

Material and Methods

We studied donor's chromosome in 36 lymphocyte cultures obtained from 70 to 80 years old 18 healthy individuals. Two cultures (intact and sodium hydrophosphate treated) were set from each individual that allowed us to compare the indices of treated cultures to their own control values. Sodium hydrophosphate at the concentration of 10^{-6} μg/ml was added to the cultures at the onset and left for the entire period of incubation (72 hrs).

Activity of ribosomal genes of acrocentric chromosomes was assessed in 900 metaphases from 10 aged individuals. The probability of argentophilic NORs and the frequency of entering satellite associations by acrocentric chromosomes either in intact or in sodium hydrophosphate treated cultures were tested by comparison of two binomials.

Polymorphism of structural C-heterochromatin. The structural **C**-heterochromatin has been examined by the method described by Fernandez et al [3]. The C-segments of chromosomes 1, 9 and 16 were compared to the short arm of chromosome 16. According to this classification results were distributed to 5 variants: a, b, c, d and e and evaluated by χ^2 .

Variability of facultative heterochromatin was evaluated based on the frequency of sister chromatid exchanges (SCEs). Short term lymphocyte cultures were used for differential staining of sister chromatids. In total, 3866 exchanges were detected in 600 metaphases obtained from 20 cultures (intact and sodium hydrophosphate - treated) of 10 old donors. Equal concentrations of 5-bromdeoxyuridine (BrdU - 7.7 μg/ml) were used for all cultures.

Results and Discussion

Transcriptional Activity of Ribosomal Genes. Human ribosomal genes are localized in secondary constrictions (NORs) in satellite stalks of acrocentric chromosomes. The ribosomal genes are involved in a key cellular processes - the protein synthesis [8]. It was revealed, that silver staining was inherent only to the NOR intensively functioning at a previous interphase, and the staining intensity corresponded to the intensity of its functioning [6,7].

The ability of acrocentric chromosomes to connect to form associations, is determined by the presence of two chromatid satellite stalks [6]. The associative activity of the strands positively correlates with the intensity of Ag-staining that, in its turn, depends on the activity of the ribosomal genes located in NORs. The absence of satellite stalks or silver staining (caused by the condensation of stalks) also testifies to the inactivation of ribosomal genes [9,10].

The data obtained from the analysis of Ag-positive NORs in intact and treated with sodium hydrophosphate lymphocytes cultures derived from old donors are given in Fig. 1 a, b. It was shown, that sodium hydrophosphate increased the frequency of Ag-positive NORs in all acrocentric chromosomes (7.23) in comparison with intact cells - (5.23) ($p < 0.001$). In particular, the frequency of Ag-positive NORs of acrocentric chromosomes, involved in associations corresponded to 2.48 per cell for sodium hydrophosphate - treated cultures significantly higher than, the corresponding index for intact cultures - 1.02 ($p < 0.001$). The frequency of associations in sodium hydrophosphate - treated cells reliably exceeds the control value for intact cultures ($p < 0.001$). It should be noticed, that sodium hydrophosphate caused equal increase of all types of associations-DD, DG and GG.

In particular, according to some reports, the peptide bioregulators and chemicals [4] induced chromosome decondensation resulting in increased transcriptional activity of nucleolar organizers. It was established, that the frequencies of Ag-positive NORs and associations depended on the condensation degree (heterochromatinization) of satellite strands.

An increase in amount and size of Ag-positive NORs, as well as in a number of involved in associations acrocentric chromosomes testifies to deheterochromatinization of satellite stalks leading to the intensification of synthesis processes due to activation of ribosomal genes in aged individuals.

Heteromorphism of structural C-heterochromatin. The results of comparative analysis of C-segment indices for sodium hydrophosphate are given for three chromosome pairs (1, 9 and 16) (Table 1).

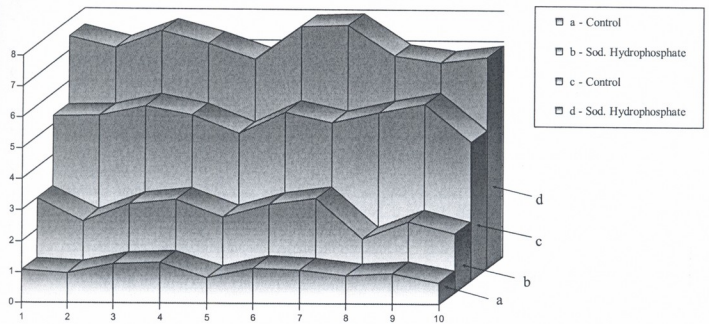

Fig. 1. Frequency of acrocentric chromosome associations and numbers of Ag-positive NORs in sodium hydrophosphate -treated cellular cultures of aged individuals. a, b - frequency of acrocentric chromosome associations; c, d - frequency number of Ag-positive NORs. On abscess axis - individuals.

Table 1. Heteromorphysm of C-bands on chromosomes 1, 9, 16 in Na_2HPO_4 (2×10^{-6}M) – treated lymphocytes from old people

Chromosomes	Variants of C-bands	ν_i	μ_i	ν_i/n	$\frac{\nu_i+\mu_i}{n+m}$	χ^2
1	a	7	10	0.0357	0.0437	χ^2_4=10,67 p<0,05
	b	60	72	0.3061	0.3393	
	c	79	84	0.4031	0.419	
	d	46	27	0.2347	0.1877	
	e	4	0	0.0204	0.0103	
9	a	29	29	0.1495	0.1543	χ^2_3=0,561
	b	95	93	0.4897	0.5	
	c	59	52	0.3041	0.2952	
	d	11	8	0.0567	0.0505	
	e	0	0	0	0	
16	a	61	52	0.3096	0.2989	χ^2_2=0,658
	b	109	99	0.5533	0.5503	
	c	27	30	0.1371	0.1508	
	d	0	0	0	0	
	e	0	0	0	0	

ν_i - number of a, b, c, d or e variants in intact cells

μ_i - number of a, b, c, d or e variants in sodium hydrophosphate - treated cells

n - total number of C-band variants in intact cells

m - total number of C-band variants in sodium hydrophosphate - treated cells

The results reflecting variability of large and small C-bands variant frequencies in sodium hydrophosphate - treated cells was reproducible. It should be noted, that the distribution of C-segment variants for chromosome 1,9 and 16 remained stable and did not differ from those for corresponding intact cells (X^2_4=10.67, p< 0.05 , X^2_3= 0.561, p> 0.05 and X^2_2= 0.658, p<0.05 - for chromosome 1, 9 and 16, respectively. As it was reported before, some chemicals are able to cause the alterations of C-bands (decrease in size) of C-bands on chromosomes 1 and 9 [4,8].

Thus, according to the obtained data sodium hydrophosphate induces decondensation of pericentromeric C-heterochromatic regions of the 1 chromosomes, however can not change in size of the 9 and 16 chromosomes C-heterochromatin.

Variability of the facultative heterochromatin studied by SCE test. In total, 2622 exchanges were registered sodium hydrophosphate - treated cells corresponding on average to 8,74±0,17 per cell, for intact cultures of the same individuals this value equalled 5,99±0,14 SCE/cell (p< 0.001).

To reveal the chromosomes responsible for increase of average SCEs on the evaluation of SCE distribution over chromosome groups was performed. The analysis showed that sodium hydrophosphate significantly increase SCE counts in A,B, D and E. No difference between the SCE indices was observed for chromosomes of C, F and G groups (p > 0.05). Therefore, the increased frequency of SCEs under the influence of sodium hydrophosphate indicates condensation (deheterochromatinization) of condensed regions. Sodium hydrophosphate, due to its ability to decondensate chromatin, favours reactivation of the genes that were repressed as a result of heterochromatinization of euchromatin regions in the process of aging.

Therefore, the results indicate, that the sodium hydrophosphate can induce reactivation of chromatin through its ability to modify the heterochromatinized chromosome regions in cells of aged individuals.

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ჰეტეროქრომატინის რეაქტივაცია ნატრიუმის ჰიდროფოსფატით მოქმედებისას ხანდაზმული ასაკის ინდივიდებში

ლეჟავა თ., ბაბლიშვილი ნ.

გენეტიკის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 28.07.2003)

რეზიუმე

შესწავლილია 70-80 წლის ინდივიდთა ქრომოსომების ფუნქციური მახასიათებლები ნატრიუმის ჰიდროფოსფატის 2×10^{-6} კონცენტრაციის (არამუტაგენური დოზა) ხსნარის მოქმედებისას. აღინიშნა ქრომოსომათა გარკვეული უბნების დეკონდენსაცია რიბოსომული გენების ტრანსკრიპციის ინტენსივობისა და შვილეულ ქრომატიდთაშორისი გაცვლების სიხშირის მიხედვით. 1-ელ, მე-9 და მე-16 ქრომოსომათა C-ჰეტეროქრომატინული ბლოკების პოლიმორფიზმის გამოვლენის საფუძველზე უპირატესად გამოიკვეთა 1-ლი ქრომოსომის სტრუქტურული ჰეტეროქრომატინის დეკონდენსაცია.

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Genetics

ANEUPLOIDY AND DIFFERENCES IN REPLICATION TIMING OF HOMOLOGOUS α - SATELLITE DNA LOCI IN LYMPHOCYTES EXPOSED BY COBALT AND CADMIUM.

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(Received July 28, 2003)

Abstract

Using Fluorescence *in situ* Hybridization (FISH) to interphase nuclei, we examined whether the pattern of centromeres replication (in the course of the S-phase) affects the key role played by centromeres (during mitosis) in segregation and their equal distribution to the daughter cells in phytohemagglutinin (PHA) - stimulated lymphocytes of the control tests and metallurgy workers. Using mono-color FISH on interphase nuclei, the replication patterns of homologous DNA loci associated with human centromeres (α - satellite sequences) of two pairs of chromosomes (10 and 17) and the rate of aneuploidy for these chromosomes in lymphocytes of various female genotypes were studied. The assay which elucidate the replication patterns of the various centric loci was based on the replication-depended configuration signals obtained at interphase following FISH with an α - satellite chromosomes – specific probe. Our data showed good correlation between the replication timing of α - satellite sequences and centromeric function: chromosome pairs whose homologous α - satellite loci replicated highly synchronously revealed low rates of aneuploidy and chromosome pairs from lymphocytes of Cadmium and Cobalt production workers, whose homologous α - satellite loci replicated asynchronously, showed the highest rate of aneuploidy.

Key words: aneuploidy, heavy metals, fluorescence in situ hybridization, α - satellite, replication.

Introduction

The aim of the work was to compare Cadmium and Cobalt influence on chromosome number, particularly on 10^{th} and 17^{th} chromosomes with the control group; also to research the type of replication of centromeric α - satellite region of 10^{th} and 17^{th} homologous chromosomes, with using of FISH. The subject of the research was peripheral blood lymphocytes of people, working in Heavy Metal (HM) manufacture. We graded the consequences of influence of HM salts on a genome material of workers with chromosomes' anomaly (aneuploidy) criteria. Worker's length of service on a manufacture was from 5 to 20 years. The character, length and form of contact with HM salts (Chlorides, Nitrites, Sulfates and etc.), particularly Cadmium (CD) and Cobalt (Co), differed in various manufactures and professions.

Mutagenic and carcinogenic effect of Cadmium and Cobalt are already proven; it's seen in losing control over cell cycle and non-stability of genome [1]. During chemical mutagenesis, caused by Cadmium and other heavy metal, carcinogenic changes and proliferation of cell are often observed, and aneuploidy is not a rare case [4, 8] In literature we have data about HM –induced genome non-stability and chromosome

aberration, which are caused by the disorganization of spindle division and appearance of different types of aberration in cancer cells of pancreas tissue [7].

The reasons and mechanisms why chromosomes don't separate during cell division and they cause aneuploidy are not known exactly. Some heavy metals and their combinations are highly active towards cell membrane and division apparatus. Certain genes control the process of cell division and regulate other stages of cell cycle. At least two stages of cell cycle are checkpoints for DNA disturbance; there are G1-S and G2-M transitions. These transitions slow down the normal development of cells. Both transitions are controlled by genetic reparation, until mutations abolish the delay [3].

Centromere complex, which consist from alfoid (heterochromatin) DNA sequences and hold up sister chromatids till anaphase, are regions, where spindle is placed (microtubule knots) and determine right segregation of chromosomes during mitosis. Heterochromatin of chromosomes and heterochromatinized regions, i.e. the regions of facultative heterochromatin, are inactivated and replicate in the end of S phase. Heterochromatin regions can greatly influence the behavior and functioning of chromosomes, also the process of DNA division and mitotic cycle [5].

The work showed that replication type and time of α-satellite DNA loci of chromosomes play a very important part in normal chromosomes segregation during cell division. In the cells, where mitotic segregation of chromosomes goes well, centromere regions of homologues pass the replication process at the same time. On the other hand, in the cell, where balanced segregation of chromosomes is destroyed, replication of homologous pares is asynchrony. This can be caused by the condition of DNA locus in the S period of cell cycle in the given type of the cell (dispyralization, connected with genetic activity or spyralization; causing repression of activity of genes). [2].

Materials and Methods

In this work the FISH (Fluorescence *in situ* Hybridization) methods utilizing chromosome – specific α-satellite probes were used. We followed two pairs of autosomes (10 and 17) in call samples of lymphocytes derived from Metallurgy workers of Cobalt and Cadmium production and in the control test. The FISH method was employed both to determine the rate of aneuploidy as well as the pattern and timing of centromeres replication.

Cell cultures. Samples of peripheral blood were obtained from 10 normal healthy females (Control group) and from 20 females, accordingly 10 of them from Cobalt (Co) production and 10 – from Cadmium (Cd) production of metallurgy. Each sample was incubated for short-term culture and prepared.

Probes. Two α - satellite DNA probes (Oncor), each specific for centromeric α - satellite region of a different chromosome, labeled with digoxigenin or biotin, were used: D10Z1 for chromosome – 10, D17Z1 for chromosome – 17. Given probes were of 200-500 Pb lengths.

Fluorescence *in situ* hybridization and signal detection. FISH was carried out according to the protocols recommended by the manufacture (Oncor). Slides were stored at -20°C until analyzed on an Olympus BH2 fluorescent microscope fitted with appropriate filter combination.

Results and Discussion

30 people were investigated: 10 workers from Cadmium metallurgy and 10 workers from Cobalt metallurgy, who accordingly had contact with Cadmium and Cobalt salts, and 10 healthy donors, who didn't have any contact with HM salts.

Specially marked α-satellite zones, that were hybridized with correspondent DNA centromere region of 10^{th} and 17^{th} chromosomes, gave fluorescence signals with the interphase nuclei of lymphocytes. With aneuploidy case instead of two signals, corresponding to homologous pair, we got one signal on the one nucleus (monosomy), three signals (trisomy), four (tetrasomy) etc.

In Table 1, we can observe the data, received after the comparison of results of common aneuploidy level in all researched groups of the experiment, which were processed with Mann-Whitney U Test. They enable us to answer the main questions in this part of experiment. There is a clear increase of common level of aneuploidy for 10^{th} and 17^{th} chromosomes in the peripheral blood lymphocytes in Cadmium and Cobalt metallurgy workers $p<0,001$, in comparison with the control group of people, having no contact with HM

salts. While comparing the data of cell types of aneuploidy in 10^{th} and 17^{th} chromosomes, there were no reliable differences in common level of aneuploidy between the data of Cadmium and Cobalt metallurgy workers (p>0,10).

Table 1. Comparison of Mann-Whitney U Test between the various groups of males in the levels of aneuploidy (NS - not significant; S - significant).

Group	Aneuploidy				
	Monosomy	Trisomy	≥	Tetrasomy	Total aneuploidy
Co v.s. Control	U=199.5 P>0.10 NS	U=52.5 P<0.001 S		U=12.0 P<0.025 S	U=74.5 P<0.001 S
Cd v.s. Control	U=169.0 P>0.10 NS	U=55.0 P<0.001 S		U=12.0 P≅0.05 ~S	U=52.5 P<0.001 S
Co v.s. Cd	U=171.5 P>0.10 NS	U=174.0 P<0.10 NS		U=220.0 P>0.10 NS	U=189.5 P>0.10 NS

P=0.001, U=88.0

Using the FISH method, we studied the dynamics of replication of 10^{th} and 17^{th} homological chromosomes α-satellite regions (high frequency of repeating alfoid DNA sequences, that located in centromere regions of chromosomes) in the peripheral blood lymphocytes of the same metallurgy workers. Information obtained from FISH is significant, because we can use the time of replication as a parameter for grading genetic activity of DNA segments [6].

Received signals of fluorescence hybridization with form of lighted dots, that correlated with 10^{th} and 17^{th} chromosome centromere fragments, had different forms on cell nucleus in the interphase stage. We found 4 types of fluorescent signals: 1) Lights, which have relatively small, round form and look like dotted fluorescence signals, called-S1; 2) Dots of lights, which look like little flurry stains, called-S2; 3) Hybridization signals, which have prolonged form, called-D1; 4) Signals of hybridization with two different double dots, called-D2 (Fig. 1).

Dynamic changes of given hybridization signals in the interphase cells occur with different frequency. Accordingly, the type of signals configuration depends on replication time. Depending on the main period of interphase, given forms of signals are ascribed to "single"-having dot-like form before replication (S1, S2) and "double"-after replication (D1, D2). It appears that, prior to replication each DNA locus showed a single, distinct dot-like hybridization signals (S1). In preparation, or in the beginning of replication the signal becomes larger, somewhat dispersed and often contains a bead-like entities (S2). Later on, at the onset of replication, the signal changes to an elongated, rod-like shape (D1). And finally, toward the end of replication it becomes a double bipartite signal (D2). Hence, following FISH with an α - satellite chromosome – specific probe, a given DNA locus undergoes in the course of replication a sequence of 4 signal configurations:

S1 → S2 → D1→ D2.

In a normal nucleus with diploid number of chromosomes, there should be two homologues for each autosome. After the cells are processed with FISH method, fluorescence zones give out two signals in accordance with homological chromosomes, in our case with 10^{th} and 17^{th} autosomes. Therefore, we received numerous nuclei of hybridization signal, similar to following types: S1S1, S2S2, D1D1, D2D2. (Fig. 1). In this case, when the morphology of signals is the same, there is synchronized replication timing between homologous chromosomes.

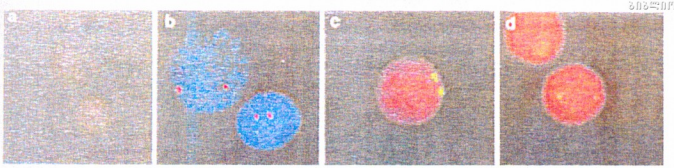
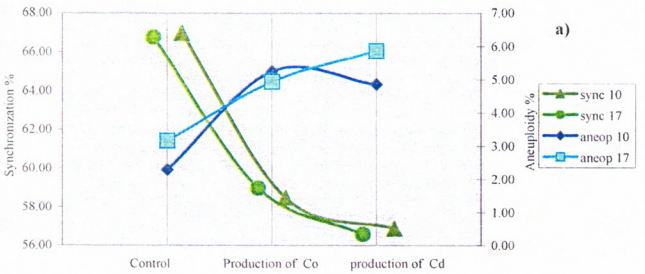


Fig. 1. Configuration of fluorescent hibridization signals identifying α-satellite chromosome-specific loci in interphase nuclei demonstrated a synchronously replicated locus of homologous. (a) a nucleus containing two single distinct signals (S1S1); (b) a nucleus containing two single dispersed signals (S2S2); (c) a nucleus containing two double, elongated rod-like signals (D1D1); (d) a nucleus containing two double bipartite signals.



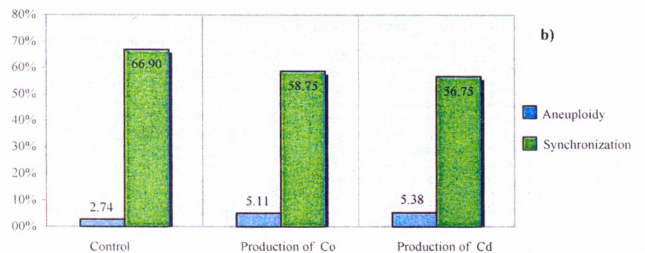


Fig. 2. a) Correlation between the level of synchronization of replication timing and aneuploidy for chromosomes 10 and 17 in the three experimental group.

b) Correlation between the common level of synchronization of replication timing and aneuploidy for chromosomes 10 and 17 in the three experimental group.

But only 60 – 70% of these kinds of nuclei, where there are morphologically alike signals and which show hybridization regions of two homological chromosomes, exist in the lymphocytes of healthy people. The remaining 30 – 40% are lymphocytes which don't have alike morphology or an asynchrony replication. In these cases we observed the following types of nuclei with pare signals: S1S2, S1D1, S1D2, S2D1, S2D2, D1D2.

In total, 4000 interphase nuclei of lymphocytes were studied in the 2nd group of workers: 2000 interphases to study the replication of centromere regions of 10th chromosomes (homologue pares) and 2000 interphases for 17th chromosome. For each case, the number of all forms of fluorescence signals for each chromosome (10 and 17) was counted separately. Meanwhile, we also marked the cases of synchronization between homologous pares of given chromosomes (S1S1, S2S2, D1D1, D2D2).

Results of synchronization of replication between homologous pairs of 10th and 17th chromosomes in the Cadmium and Cobalt metallurgy workers compared to the control group, demonstrate, that percentage of cells, where homologous pairs of chromosomes 10 and 17 pass the synthesis of DNA in the same way (i.e. are kept in time), is decreased in the Cd and Co metallurgy workers, compared to the data of healthy donors

In healthy donors (the group consists of 10 people at the age of 25-50 years) centromere regions of 10th and 17th chromosomes in lymphocytes pass the process of replication synchronically between the homologous pairs, in 62-71% cases, and in 29-38% cases it is asynchrony.

Accordingly to the data we received in lymphocytes of people, who don't have any contact with metallurgical Cadmium and Cobalt, homologous pairs of chromosomes (centromere regions of 10th and 17th chromosomes) replicate synchronously, with average level of synchronization: 66,90±0,82%; common level of aneuploidy is 3,12±0,41%. In workers who contact with metallurgical Cobalt, average level of synchronization is lower: 58,75±0,93% and the average level of aneuploidy is higher: 5,11±0,50%. In workers contacting with metallurgical Cadmium, average level of synchronization has been lowered up to: 56,75±1,07%, and average level of aneuploidy is high: 5,38±0,37%. Increase of aneuploidy level, like trisomy, tetrasomy and more than tetrasomy, in both (Cadmium and Cobalt) groups of workers is higher than in the control group of the experiment $p<0,001$. (Fig. 2).

Thus, our study confirms that because centromeres control integration and stability during cell division losing balance and concordance of their function (that is expressed in losing of synchronic replication), can cause chromosomes not to separate or even lose genetic material during cell division. This causes appearance of chromosome aberration like monosomy, trisomy, tetrasomy etc.

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კობალტისა კადმიუმის მოქმედებით გამოწვეული ანეოპლოდიისა და α-სატელიტური დნმ – ლოკუსების რეპლიკაციის დინამიკის დამოკიდებულება.

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(მიღებულია 28.07.2003)

რეზიუმე

FISH მეთოდით მიღებული ქრომოსომათა ცენტრომერული უბნების ფლუორესცენტული ნათების წერტილების კონფიგურაციის მიხედვით დადგენილ იქნა დნმ-ის რეპლიკაციის დინამიკა და თანამიმდევრობა (S1 – რეპლიკაციამდე; S2, D1, D2 – რეპლიკაციის მიმდინარეობის დროს), რის მეშვეობითაც განისაზღვრა ჰომოლოგიურ ქრომოსომათა რეპლიკაციის სინქრონიზაციის ხარისხი.
დადგენილ იქნა, რომ მე-10 და მე-17 ქრომოსომათა ჰომოლოგიური წყვილების ცენტრომერული უბნები, ლიმფოციტებში რეპლიკაციის სინქრონულად გადიან. მძიმე მეტალებით გამოწვეული ქიმიური ზემოქმედება არღვევს ჰომოლოგიური ქრომოსომების მოცემული უბნების გაორმაგების პროცესის სინქრონულობას. გამოვლენილია უარყოფითი კორელაცია რეპლიკაციის სინქრონიზაციასა და ანეუპლოიდიის სიხშირეს შორის კადმიუმის და კობალტის ზემოქმედებისას.

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A CYTOGENETIC STUDY OF MENTALLY RETARDED CHILDREN

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Abstract

12 children with undifferentiated forms of mental retardation (patients' age 10-15 years old; the degree of mental retardation in the main is within the range of debility) were cytogenetically studied. The following parameters were evaluated: structural and quantitative chromosome disorders; transcriptional activity of nucleolus organizing regions (NORs) in acrocentric chromosomes. 6 patients have significantly increased level of chromosome instability. The frequency, size and associative activity of Ag^+-NORs were decreased.

Key words: mental retardation, chromosomal disorders, acrocetric chromosomes, nucleolar organizing region.

In accordance with the literature data various types of chromosome abnormalities present among mentally retarded (MR) patients with much more frequency than among general population [4-6]. It concerns both general chromosome instability and particular cases of chromosome abnormalities. At the same time the results represented by different authors vary over a wide range in different countries. Last years' data allow to consider some chromosome types as the reason of mental retardation [1-8]. The above stated indicates the necessity of further investigations in this direction.

The work presents a cytogenetic study of 12 children with undifferentiated forms of mental retardation (patients' age 10-15 years old; the degree of mental retardation in the main is within the range of debility). The following parameters were evaluated: stability of chromosome pool by frequency of structural and quantitative chromosome disorders, and the level of synthesic processes by assessment of transcriptional activity of nucleolus organizing regions (NORs) of the acrocentric chromosomes (segments, located on the satellite filaments of acrocentic and containing clusters of ribosome genes).

Material and methods

Chromosomes mutation and transcriptionally active NOR of acrocentric chromosomes have been studied in 12 children with undifferentiated forms of mental retardation.

As the material for investigation cells of phytohemagglutinin (PHA) – stimulated lymphocyte cultures of peripheral blood (the RPMI – medium was used as the culture medium) were taken. Cultivation of lymphocytes, preparation and staining of chromosome slides were carrying out by standard method. During evaluation of quantitative and structural chromosome disorders of every patient at least 150 metaphases were analyzed. To reveal transcriptionally active NOR of acrocentric chromosomes the method of silver

impregnation (Ag-banding) was used. The sizes and quantity of satellite filament silvered segments and the frequency of their joining in associations were taken into account. The sizes of silvered segments were assessed by 2-point scale: 1 – small segments (less than chromatic width); 2 – large (equal to or more than chromatic width) [3]. From each patient 30-50 metaphases with silver stained acrocentic chromosomes were analyzed.

Results and discussion

As a result of analysis of quantitative and structural chromosome disorders in 6 patients (50% of examined ones) were found to have significantly increased frequency of cells with structural chromosome rearrangements (this index varied among patients within the range of 4.50±1.46% to 7.50±2.08%), among other 50% significant differences with the mean control level (1.67±0.42) for patients with normal mentality (NM) were not found.

These results are in agreement with reference to the data indicating increased level of chromosome instability for various forms of oligophrenia [1, 3, 5], although instability index in our investigations is higher.

As for structural chromosome rearrangements significant prevalence of single and pair fragments was found; less frequently chromosome and chromatide translocations were registered. It should be noted that for one patient with oligophrenia in 15% of examined metaphases C-group chromosome with enlarged secondary constriction of a long arm was found. As it was reported different types of oligophrenia are characterized by the presence of various marker chromosomes, in the main X, 2, 8, 9, 16, 19 and Y, which some authors consider to be a causative factor of some forms of mental retardation [4, 6, 7]. In our study the marked chromosome was presented by only one homologue of a pair.

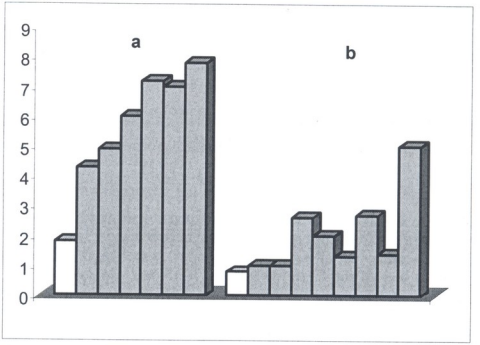

Fig. 1. Individual variability of the frequency of cells with chromosome aberrations (a) and polyploid cells (b) in the children with MR having high levels of these indices. The numbers indicate tested individuals.

- mean control index for the donors with NM, - indices for the donors with MR.

As for quantitative chromosome disorders it was found that the frequency of aneuploid cells among all the examined patients did not differ from the mean control index. In our study the average frequency of aneuploid cells among mentally retarded children turned to be 6.93±0.56% (the mean control index 6.0±0.75%), in both cases the cells with hypodiploid chromosome sets were in prevalence. Analysis of polyploidy has revealed quite different results. It was found that among 8 children with oligophrenia (66% of examined patients) the frequency of polyploid cells (the limits of variations among the patients are 1.0%-

5.0%) is significantly increased in comparison with mean control (0.02±0.01%) that admits to suggest more high frequency of disorders in mitosis among this group of mentally retarded children.

As it has been already noted along with investigation of chromosome instability we have performed the analysis of transcriptional activity of NOR of acrocentric chromosomes during no-ndiferentiated form of oligophrenia using the method of silver staining. For this purpose 7 mentally retarded children were examined. As it is known only those nucleolus organizers are stained by silver that contain the ribosome genes actively transcribing in previous interphase.

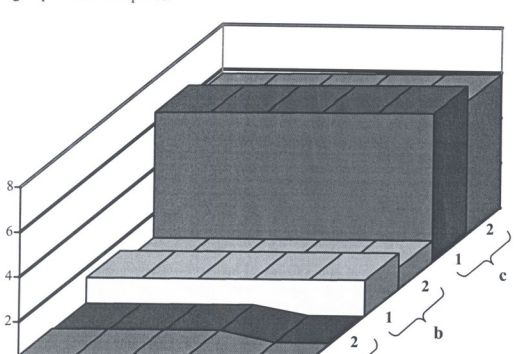

Fig 2. Average frequency per cell

a. Associations of acrocentric chromosomes: 1.Normal (N); 2.Mentally Retarded(MR).
b. Ag+ -NORs of different sizes for in: 1.N; 2.MR.
c. Large Ag+ -NORs (2 scores): 1.NM; 2.MR.

The frequency, size and associative activity of Ag – positive segments of satellite acrocentric stalks are important characteristics of cell functional state and their synthesis activity level. In all studied 305 cells it turned out that the mean frequency of all Ag – positive acrocentrics (13-, 14, 15-th pair of the D-group homologues and 21-, 22-nd pair of the G-group) per cell is slightly decreased among the patients with oligophrenia (5,81±0.13) in comparison with the mean control index (6.5±0.11) (Fig. 2). The relation between characteristics of silvered nucleolus organizers per cell for chromosomes of D and G groups among mentally retarded patients was the same as in control case. The analysis of large (2-scale) silvered segments has found that their total mean frequency for both chromosome groups per cell (0.92±0.05) among mentally retarded children is significantly less than the mean control level (1.56±0.05). The relation between characteristics of Ag^+ – segments for D and G acrocentric chromosomes per cell was also in agreement with control index. The analysis of acrocentric chromosome associative activity shows that for mentally retarded children the decreased mean level of this index is observed (0.42±0.03) in comparison with the mean control index (0.49±0.03). Decrease in associative activity of NORs in acrocentrics at oligophrenia is also indicated in other publications [8, 9]. As for the frequency of occurrence of different association types (DD, DG, GG) the ratios between averaged characteristics among children with oligophrenia does not differ from the control one (Fig. 2), nevertheless it should be noted that this parameter is characterized by significant variability among patients.

Summarizing the results of investigation of active nucleolus organizing acrocentrics and of their associative ability, we can conclude that the transcriptional activity of nucleolus organizers among examined

patients' group with non-differentiated form of oligophrenia is decreased in comparison with the control index for individuals having normal mentality. The above mentioned indicates decreased level of synthesic processes in cells of examined mentally retarded children which in turn, in accordance with the reported data is approved by decreased cell reparative processes and thus must be the reason for increased level of chromosome instability revealed by us.

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გონებრივად ჩამორჩენილ მოზარდთა ციტოგენეტიკური შესწავლა.

დადუნაშვილი ე., ჯოხაძე თ., ტაბატაძე ნ.

გენეტიკის კათედრა, ი. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 28.07.2003)

რეზიუმე

ჩატარებულია გონებრივად ჩამორჩენილი 12 მოზარდის (ასაკი 10-15 წელი, გონებრივი ჩამორჩენილობა დებილობის ფარგლებში) ციტოგენეტიკური გამოკვლევა. შესწავლილია ქრომოსომათა სტრუქტურული და რაოდენობრივი დარღვევების სიხშირე და აკროცენტრულ ქრომოსომათა ბირთვაკვარმომქმნელი რაიონების (ბზრ) ტრანსკრიფციული აქტივობა. ნაჩვენებია, რომ ინდივიდთა 50%-ს ახასიათებს ქრომოსომული აპარატის არასტაბილურობის მაღალი დონე. აკროცენტრულ ქრომოსომათა აქტიური ბზრ სიხშირე, ზომები და ასოციაციური აქტივობა გამოკვლეულ გონებრივად ჩამორჩენილ მოზარდებში დაქვეითებული აღმოჩნდა.

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THE EFFECT OF MERCURY IONS ON THE FREQUENCY OF STRUCTURAL AND NUMERICAL CHROMOSOME ABNORMALITIES IN HUMAN

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Abstract

The effect of solutions with several concentrations of Mercury-chloride (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}M) on the frequency of structural and numerical chromosome abnormalities with anticipation an individual susceptibility of chromosome apparatus have been studied in short-term lymphocyte cultures of 20-30 year age individuals. The dose-dependent effect of tested salt has been shown. Individual variability of studied parameters has been indicated at the low concentration of Mercury-chloride.

Key words: Mercury-chloride, chromosome, aberration, aneuploidy.

Introduction

Mercury salts represent the compounds which are wide-spread in environment and which mutagenic activity is established for many object especially for human [3, 7, 8]. It's known that they induce both gene and chromosome mutations and characterize the dose-dependent effect [1, 2, 4, 5]. On the other hand there are data which refer that the frequency of mutations induced by similar concentrations of Mercury ions considerably vary depending on individual genotypical characteristics [6, 8].

Thus, mutagenic effect investigation of solutions with several concentrations of Mercury-chloride with anticipation an individual reaction is of interest.

The aim of the present research was to study the effect of solutions with several concentrations of Mercury-chloride on the frequency of structural and numerical chromosome abnormalities with anticipation the individual features in cultured cells of human peripheral blood.

Material and methods

Investigations have been carried out on human peripheral blood lymphocyte cultures obtained from 6 clinically healthy persons at the age 20-30. Lymphocytes were cultivated by the standard method. Mercury-chloride solutions (at concentrations 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}M) were tested for each individual. The solutions at above mentioned concentrations were added to the cultures on the 24th hour and left for the entire period of incubation (72 hrs.). The structural and numerical chromosome abnormalities were registered on the chromosome preparations. For each individual the results were compared with the own control values.

Table 1. The impact of $HgCl_2$ on the levels of chromosome aberration and aneuploidy

do-nors	Control		$HgCl_2$ 10^{-3}M		$HgCl_2$ 10^{-4}M		$HgCl_2$ 10^{-5}M		$HgCl_2$ 10^{-6}M		$HgCl_2$ 10^{-7}M	
	cells with chromos. aberrat. %±m	cells with aneuplo-idy %±m	cells with chromos. aberrat. %±m	cells with aneuplo-idy %±m	cells with chromos. aberrat. %±m	cells with aneuplo-idy %±m	cells with chromos. aberrat. %±m	cells with aneup-loidy %±m	cells with chrom. aberrat. %±m	cells with aneuploi dy %±m	cells with chrom. aberrat. %±m	cells with aneup-loidy %±m
1	1,0±1,0	5,0± 2,2	14,0± 3,7	16,0± 4,0	12,0±3,4	14,0± 3,7	5,0±2,2	13,0±3,6	3,0±1,7	10,0±3,1	1,0±1,0	6,0±2,4
2	3,0±1,7	7,0±2,6	18,0±4,2	18,0±4,2	15,0±3,8	16,0±4,0	10,0±3,1	13,0±3,6	7,0±2,6	11,0±3,3	4,0±2,0	9,0±3,0
3	2,0±1,4	8,0±2,8	15,0±3,8	19,0±4,3	13,0±3,6	17,0±4,1	11,0±3,3	14,0±3,7	7,0±2,6	12,0±3,4	3,0±1,7	10,0±3,1
4	3,0±1,7	5,0±2,2	14,0±3,7	13,0±3,6	9,0±3,0	11,0±3,3	7,0±2,6	9,0±3,0	5,0±2,2	8,0±2,8	4,0±2,0	6,0±2,4
5	2,0±1,4	7,0±2,6	16,0±4,0	15,0±3,8	14,0±3,7	13,0±1,6	9,0±3,0	10,0±3,1	6,0±2,4	9,0±3,0	3,0±1,7	7,0±2,6
6	2,0±1,4	7,0±2,6	17,0±4,1	14,0±3,7	12,0±3,4	11,0±3,3	8,0±2,8	9,0±3,0	6,0±2,4	8,0±2,8	4,0±2,0	7,0±2,6
total	2,1± 0,6	6,5±1,04	15,6±1,6	15,8±1,6	12,5±1,4	13,6±1,5	8,3±1,2	11,3±1,4	5,7±0,9	9,6±1,3	3,1±0,7	7,5±1,1

Results and discussion

In total 3600 metaphases were analysed for establishing structural and numerical chromosome abnormalities induced by solutions with several concentrations of Mercury-chloride. 100 metaphases were analyzed for each variant of experiment and for each individual. Frequency increasing of structural and numerical chromosome abnormalities was detected in all individuals according to elevation of tested salt concentration (Table 1). Common chromosome damages were single and paired fragments. The results confirm the published data. The lowest value of chromosome aberrations by individuals was fixed at concentration 10^{-7}M. The total frequency of cells with chromosome aberrations was 3,1% and that didn't reliably differ from the control value (2,1%). The frequency of aberrant cells reached maximum value at the concentration 10^{-3}M. According to these parameters most significant differences were detected between the values at concentration 10^{-3} M and 10^{-5} M also 10^{-5} M and 10^{-7}M. Individual variability of aberrant cell frequency according to concentrations was 10^{-3}M- 14-18%; 10^{-4}M - 9-15%; 10^{-5}M - 5-11%; 10^{-6}M - 3-7%; 10^{-7}M - 1-4%. Thus, individual variability was most visible for solutions at low concentrations.

The same evidences were fixed for variability of aneuploidy frequency. The dose-dependent effect was detected for this parameter. Aneuploidy frequency was increased according to the concentration elevation. Total frequency of cells with aneuploidy was minimal at concentration 10^{-7}M (7,5%) and that didn't reliably differ from control value (6,5%). It must be noted that some individual variability according to this value was detected at the low dose ranges (10^{-5}M, 10^{-6}M, 10^{-7}M).

Thus, there were detected some specificity expressing in individual variability of structural and numerical chromosome abnormality frequency, especially during low concentration solutions exposure by Mercury-chloride ions.

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ვერცხლისწყლის იონების გავლენა ადამიანის ქრომოსომათა სტრუქტურული და რაოდენობრივი დარღვევების სიხშირეზე

ჯოხაძე თ., თადუმაძე ნ., ბაბლიშვილი ნ.

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რეზიუმე

შესწავლილია ვერცხლისწყლის ქლორიდის სხვადასხვა კონცენტრაციის (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}M) ხსნარების გავლენა ადამიანის ქრომოსომათა სტრუქტურული და რაოდენობრივი დარღვევების სიხშირეზე 20-30 წლის ინდივიდთა სისხლის ლიმფოციტთა მოკლევადიან კულტურებში ქრომოსომული აპარატის ინდივიდუალური მგრძნობელობის გათვალისწინებით. ნაჩვენებია ამ მარილის დოზა-დამოკიდებული ეფექტი, გამოვლენილია შესასწავლი პარამეტრების გარკვეული ინდივიდუალური ვარიაბელობა ტესტირებული მარილის დაბალი კონცენტრაციის ხსნარებით ზემოქმედებისას.

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GENOME CHARACTERISTICS AT CONNECTIVE TISSUE DISEASES - SYSTEMIC LUPUS ERITHEMATOSUS AND SYSTEMIC SCLERODERMA

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Abstract

To define the proliferative activity of lymphocytes blasttransformative activity to mitogen phytohemagglutinin (PHA) in blood lymphocyte cultures of patients with systemic lupus erythematosus (SLE) and systemic scleroderma (SSD) was studied. In the case of SLE data were lower than in the control group, but with SSD it was higher. Studies of dynamics of cell division showed, that in cultures cell populations are highly asynchronic. There was no correlation between mitotic index and percentage of aberrant cells. Chromosome aberrations were increased both in SLE and SSD.

Key words: Systemic Lupus Erythematosus, Systemic Scleroderma, Lymphocyte, proliferation, dynamics of cell division, chromosome aberrations, aneuploidy, polyploidy.

Introduction

Systemic diseases of connective tissue occur frequently and it consists of many groups of pathologies. Each of them characterized by chronic and progressive currency has an autoimmune nature [4] and belongs to the pathologies of DNA reparation-replication processes [1,5,7]. Despite the fact that the etiology of the disease is not known clearly, many researches show the important role of genetic factors in its pathogenesis. There is a clear hereditary predisposition to the development of the connective tissue diseases [2,5]. The main genetic researches of these pathologies are clinical-genealogic and immunogenetic [1]. Patient's individual genetic heterogeneity defines the clinical polymorphism of the disease. So, the study of the morpho-functional rates of chromosomes in the SLE [3] and SSD cases is reasonable. Our goal was to study proliferative activity and chromosome instability in lymphocytes from the patients with SLE and SSD.

Material and methods

The peripheral blood lymphocyte cultures of 19 individuals (the patients with SLE and SSD, and healthy donors) were studied. Patients were diagnosed and tested at the Republican Rheumatologic Scientific-Practice Center of Georgia. Proliferative activity of lymphocytes of patients with SLE and SSD were graded with two parameters: the percentage of blast cells to mitogen phytohemaglutinin (PHA) and the dynamics of mitotic cycle using differential staining of sister chromatides [6]; We added the analogue of thymidin—5 Bromdeoxyuridine (5-bromo-2-deoxyuridine, Sigma) in 7.7 mg/ml concentration in the tested culture. On the fourth day after fixation of cultures we irradiated them with special uv - lamp and stained with azur-eozinum, then determined proliferative index with the following formula:

$$\text{Proliferative index:} = \frac{\sum_{i=1}^{n} (iAi)}{\sum_{i=1}^{n} Ai}$$

i – number of mitosis, Ai – number of cells being at i - mitosis

To study the correlation between these data and cytogenetic characteristics, we determined the frequency of chromosome aberrations and aneuploid and polyploid cells. Short-term cell cultures were used. The patients with SLE were divided in two groups: I - the untreated individuals and II - the patients with chronic disease, that have been treated before. Student's criterion was used to study and count the data (Table).

Results and discussion

Blasttransformative activity. An average level of blasttransformative activity in healthy individuals was 56.30±0.50%. In treated patients with SSD these data were significantly increased - 72.60±0.99%. It is interesting that in both (I, II) groups of patients with SLE, data were significantly lowered compared to the control group - 32.87±0.86% and 33.27±0.50% respectively [3].

Dynamics of cell division. We studied the dynamics of cell cycle in culture to observe the proliferative activity of stimulated lymphocytes by PHA. We measured the percentage correlation of cells in the 1st, 2nd, 3rd, 4th mitosis, obtained from the differential 72 hour cultures of lymphocytes. Analyses showed that on the 72nd hour of incubation in all studied cultures, cell populations were highly asynchronic. In the case of SLE the percentage of the cells in the 1st and 3rd stage mitosis didn't differ from the control group data. Most of the cells (control and I,II groups) were in the 2nd mitosis cycle. There was no correlation between the percentage data of mitotic index and the aberrant cells.

Chromosome dysfunction. As for the spontaneous chromosome dysfunctions (correlation with control group 1.70±0.42%) in SLE a high frequency of dysfunction (12.33±1.90% and 11.89±1.07%) was found. In all these patients single and pair fragments were dominant. Symmetric chromatid and asymmetric chromosome translocations, non-centered circles, torn fragments in centromere and early separation of centromeres were revealed.

In both SSD and SLE cases, aberrant metaphases (10.53±2.23) and aberrations were increased compared to the control group [1]. Aberrations were from single and pair fragments. Also, decentral chromosomes and single cases of translocations were revealed. Content of aneuploid cells was lowered compared to the control group in the patients with SLE; and in SSD case there data were increased (3.16±1.27%), but in polyploid cells - slightly increased (1.58±0.90%).

In both forms of disease our results accord to the data presented in the literature regarding dysfunction of reparation mechanisms and diseases caused by damaged genetic material [1,5].

Table. Cytogenetic charachteristics of patients with SLE and SSD

Desease's forms	The blast cells	Number of metaphases	The aberrant cells	Aneuploidy		Polyploidy
				hipo-	hyper-	
SLE						
I	32,87+0,86	300	12,33+1,90	0	0	0
II	33,27+0,50	917	11,89+1,07	1,20+0,36	1,20+0,36	0,22+0,15
SSD						
II	72,60+0,99	200	10,53+2,23	3,16+1,27	0	1,58+0,90
Control						
10 Don.	56,30+0,50	930	1,70+0,42	1,94+0,45	0,33+0,19	0,43+0,07

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შემაერთებელქსოვილოვანი დაავადებების ზოგიერთი ფორმის (სისტემური წითელი მგლურა და სკლეროდემია) ციტოგენეტიკური მახასიათებლების შესწავლა

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(მიღებულია 28.07.2003)

რეზიუმე

ლიმფოციტების პროლიფერაციული აქტივობის განსაზღვრისათვის შესწავლილ იქნა სისტემური სკლეროდერმიით (სკდ) და სისტემური წითელი მგლურით (სწმ) დაავადებული პაციენტების სისხლის ლიმფოციტების კულტურებში ბლასტ-ტრანსფორმაციული აქტივობა მიტოგენ ფიტოჰემაგლუტინინის (ფჰა) მიმართ. სწმ-ს შემთხვევაში აღნიშნული მაჩვენებელი საკონტროლოსთან შედარებით დაქვეითებული, ხოლო სკდ-ს შემთხვევაში კი მომატებული აღმოჩნდა. უჯრედული ციკლის დინამიკის ანალიზმა აჩვენა, რომ კულტურებში უჯრედული პოპულაციები მაღალი ასინქრონულობით ხასიათდებიან; მიტოზურ ინდექსსა და აბერანტული უჯრედების პროცენტულ მაჩვენებლებს შორის კორელაცია არ იქნა გამოვლენილი. ქრომოსომული დარღვევები მომატებული იყო როგორც სწმ-ს, ასევე სკდ-ს დროს.

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Genetics

THE MUTAGENIC EFFECT OF HEAVY METAL SALTS ($PB(NO_3)_2$, $CDCL_2$ AND $NICL_2$) ON DROSOPHILA

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Abstract

The mutagenic effect of three compounds: $Pb(NO_3)_2$, $CdCl_2$ and $NiCl_2$ (three doses for each: 10^{-1} M, 10^{-2} M and 10^{-3}M) has been studied in Drosophila to register sex-linked recessive lethal and sublethal mutations. All the three compounds revealed different levels of mutagenic activities in a dose-dependent manner, but $Pb(NO_3)_2$ was the most effective. Besides the mutagenic effect, the studied metals revealed to have some physiological effect: three categories of wing morphosis– long-winged, single-wing-opened and double-wing-opened ones were found in generations of treated flies.

Key words: Drosophila, heavy metals, mutations, morphosis.

Introduction

In recent years concentration of heavy metal salts significantly increased in environment. Metals accumulate in living organisms, in both- pro- and eukariotic systems and exhibited their mutagenic and carcinogenic activities [7].

Most metal compounds are able to dissociate and release bevalent cations (Co^{2+},Zn^{2+},Ni^{2+},Mn^{2+},Cd^{2+}, etc.), directly interacting with DNA, others contain the elements of variable valency (such as – Mo, Hg, Cu, Cr, etc.) respectively inducing oxidative damages in DNA [2].

Material and methods

The goal of the presented work was to study genetic effect of some heavy metal salts in Drosophila. The standard method of "Meller-5" was applied to register frequencies of sex-linked recessive lethal and sublethal mutations. Three compounds-$Pb(NO_3)_2$, $CdCl_2$ and $NiCl_2$, three doses for each (10^{-1} M, 10^{-2} M, 10^{-3}M) were tested. The exposition time lasted for 24h. The standard nutrient medium served as a solvent.

Results and discussion

The analysis of obtained data (see Table 1) showed that all three compounds revealed the different levels of mutagenic activity in a dose-dependent manner. The most effective of the three was $Pb(NO_3)_2$ at a dose of 10^{-1} M (6.6%-lethal and 9.43%-sublethal, total- 16.03% mutations). The 10^{-2} M and 10^{-3}M solutions of $Pb(NO_3)_2$ also had mutagenic effect (the total numbers of mutations were 12.32% and 8.69%, respectively). These data are in agreement with the published data concerning mutagenic activities of lead and lead

compounds. It was stated that they were able to produce covalent bonds with nucleic acid phosphates and could induce single-strand breaks or alkali-labile sites in DNA [3].

$CdCl_2$ revealed the lower mutagenic effect in comparison to $Pb(NO_3)_2$. In the case of exposition to 10^{-1} M $CdCl_2$ the total number of mutations equaled 13.66%, for 10^{-2} M solution-10.20% and for 10^{-3}M-6.51%. It was found, that cadmium and its compounds have direct and indirect genotoxic effects through the direct induction of DNA lessions or via inhibition of excision repair mechanisms. Cd induces deletions (as a result of an oxidative stress in yeast *Saccharomyces cerevisiae* (6)); gene mutations, expressed as frame shift mutations and miscens mutations [8]. Transversions were discribed more frequently than transitions [1].

Table 1. The frequency of sex-linked recessive lethal and sublethal mutations in Drosophila after treatment with some heavy metal salts

compound and concentration	invest. crom. numb.	mutation frequency					
		lethal		sublethal		total	
		n	%±m	n	%±m	n	%±m
$Pb(NO_3)_2 10^{-1}$ M	212	14	6.60±1.71	20	9.43 ±2.00	34	16.03± 2.52
$Pb(NO_3)_2$ 10^{-2} M	219	9	4.10±1.34	18	8.22± 1.86	27	12.32± 2.22
$Pb(NO_3)_2$, 10^{-3}M	230	7	3.04 ±1.13	13	5.65 ±2.38	20	8.69± 1.86
$CdCl_2 10^{-1}$ M	227	12	5.29±1.48	19	8.37 ±1.84	31	1.66± 2.28
$CdCl_2 10^{-2}$ M	245	10	4.08 ±1.26	15	6.12± 1.53	25	1.20± 1.93
$CdCl_2 10^{-3}$M	215	6	2.79 ±1.12	8	3.72± 1.29	14	6.51± 1.68
$NiCl_2 10^{-1}$ M	234	10	4.27± 1.32	17	7.26 ±1.70	27	1.53 ±2.09
$NiCl_2 10^{-2}$ M	227	8	3.52± 1.22	14	6.17 ±1.60	22	9,69 ±1.96
$NiCl_2 10^{-3}$M	231	4	1.73 ±0.86	7	3.03 ±1.13	11	4,76 ±1.40
control	246	0	0	0	.0	0	0

Table 2. The frequency of wing morphosis in Drosophila after treatment with some heavy metal salts

compound and concentration	invest. indiv. numb.	morphosis frequency							
		long-winged		single-wing-opened		double-wing-opened		total	
		n	%±m	n	%±m	n	%±m	n	%±m
$Pb(NO_3)_2 10^{-1}$ M	4240	71	1.67±0.19	16	0.38±0.09	13	0.31±0.09	100	2.36±0.23
$Pb(NO_3)_2$ 10^{-2} M	4380	63	1.44±0.18	9	0.21±0.09	11	0.25±0.08	83	1.89±0.21
$Pb(NO_3)_2 10^{-3}$M	4600	36	0.78±0.13	23	0.50±0.11	31	0.67±0.12	90	1.96±0.21
$CdCl_2 10^{-1}$ M	4540	17	0.74±0.13	8	0.18±0.06	43	0.95±0.14	68	1.49±0.18
$CdCl_2 10^{-2}$ M	4900	12	0.24 ±0.07	20	0.41± 0.09	34	0.69±0.12	66	1.35±0.16
$CdCl_2 10^{-3}$M	4300	11	0.26 ±0.08	17	0.40±0.09	13	0.31±0.08	41	0.95±0.15
$NiCl_2 10^{-1}$ M	4680	11	0.24±0.07	9	0.19±0.06	6	0.13±0.05	26	0.56±0.11
$NiCl_2 10^{-2}$ M	4540	8	0.18± 0.06	10	0.22±0.07	4	0.09±0.04	22	0.49±0.10
$NiCl_2 10^{-3}$M	4620	4	0.09 ±0.04	2	0.04±0.02	3	0.06±0.03	9	0.19±0.06
control	4920	1	0.02± 0.02	0	.0	1	0.02±0.02	2	0.04 ±0.02

$NiCl_2$ revealed the lowest mutagenic effect-10^{-1} M solution induced 11.53% mutations, 10^{-2} M – 9.69% and 10^{-3}M – 4.76%. Our results correspond to the published data about the nickel's high carcinogenicity and lower mutagenicity compared to Cd [8]. The mutagenic effect of Ni is attributed to the DNA methylation process and chromatin condensation [4, 7] or to the production of free radicals and alkylative DNA bases [5].

Besides the mutagenic effect, the physiological effect of the compounds has been examined. Three categories of wing morphosis (long-winged, single-wing-opened and double-wing-opened) that were phenotypicaly identical to the following mutations: gull, warped and divergent respectively, have been registered in our experiment (see Table 2). The tested compounds caused induction of wing morphoses in

different frequencies. In the case of $Pb(NO_3)_2$ the highest number of morphosis has been revealed. It was found that the agent could cause induction of long-winged morphosis more frequently than the other ones (1.67%, 0.38% and 0.31% - respectively). $CdCl_2$ and $NiCl_2$ also induced morphosis of the same type, but in lower number. No significant difference in their frequencies was found.

Proceeding from our results we come to the conclusion that all the three studied metal compounds revealed mutagenic and physiological effects in Drosophila that were registered as sex-linked lethal mutations and wing morphosis.

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ზოგიერთი მძიმე მეტალის ($Pb(NO_3)_2$, $CdCl_2$ და $NiCl_2$) მარილის ზემოქმედება დროზოფილას გენეტიკურ აპარატზე

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გენეტიკის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 28.07.2003)

რეზიუმე

ჩვენს მიერ შესწავლილ იქნა ზოგიერთი მძიმე მეტალის მარილის – $Pb(NO_3)_2$, $CdCl_2$ და $NiCl_2$ (თითოეული სამ-სამი დოზით – 10^{-1} M, 10^{-2} M და 10^{-3}M) მუტაგენური ეფექტი დროზოფილაზე. სამივე ნივთიერებამ გამოავლინა სხვადასხვა ხარისხით გამოხატული დოზა-დამოკიდებული მუტაგენური ეფექტი. ყველაზე ძლიერი აღმოჩნდა $Pb(NO_3)_2$, ხოლო ყველაზე სუსტი – $NiCl_2$. საანალიზო ნივთიერებებმა მუტაგენური ეფექტის გარდა გამოავლინეს გარკვეული ფიზიოლოგიური ეფექტი ფრთის მორფოზების ინდუქციის თვალსაზრისით. ექსპერიმენტში აღირიცხა სამი კატეგორიის ფრთის მორფოზები: გრძელფრთიანი, ცალფრთაგაშლილი და ორფრთაგაშლილი.

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CHROMOSOME FUNCTIONAL STABILITY AT HYPOTHYREOIDISM ASSOCIATED WITH THYROID HYPOPLASIA

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Abstract

A patient with hypoplastic thyroid glands has been examined for chromosome stability and transcriptional activity of ribosomal cistrons before and after medical treatment. Severe chromosome disorders including polyploid cells with chromosome fragmentation and low rate of transcriptional activity were registered during the first examination. After passing a three – month course of hormonal-therapy (with levothyroxin) patient's clinical state and biochemical indices have been normalized and the cytogenetic characteristics tend to come nearer to control values.

Key words: thyroid hypoplasia, chromosome aberration, nucleolus organizing regions, Ag-positive acrocentric chromosome, chromosome association.

Introduction

Thyroid hormone synthesis requires a normally developed thyroid gland, a properly functioning hypothalamic – pituitary – thyroid axis, and sufficient iodine intake. Hypoplasia of the gland may be caused by developmental defects, bioinactive thyrotropin or resistance to thyrotropin at the level of the receptor or its signaling pathway [1, 3]. Hormonal disbalance in organisms are somehow reflected upon immunocompetent cells, in particular, on genome functional parameters of lymphocytes. Alterations of the latter depend on the form, duration and severity of disease. Proceeding from this, the monitoring over cytogenetic parameters together with biochemical indices would help the physicians to control the patient's state.

Material and methods.

The patient D. B., male, 13-years old, was diagnosed in the Republican Central Clinic of Georgia for the form of hypothyroidism associated with thyroid hypoplasia. The blood samples were taken before and after a three – month - course of treatment (with levothyroxin) and cultured for 72 hrs according to the standard method. For studying the frequency of polyploidy 1000 cells were analyzed. Silver staining of preparations was performed as recommended by Bloom and Goodpasture [2]. Number and size of Ag-positive (silver-stained) nucleolus organizing regions were visually assessed using a scoring system by Lezhava [4]. Acrocentric associations were defined as intersatellite bond identifiable by Ag-staining. 10 young healthy individuals of the same age group made up a control group.

Results and discussion.

A mean percentage of aberrant cells and a mean number of chromosome aberrations before treatment of the patient were significantly higher (16.2±4.02) than the corresponding values for the same individual after treatment (5.0±2.24) and in control subjects (1.7±0.42). The analyses of aberration types showed that common chromosome damages were single and pair fragments.

High incidence of polyploid cells was observed in cultured lymphocytes of the patient when examined for the first time - 1.3±0.36%. A major mechanism of polyploid cell production in humans is endomitosis or chromosome set duplication without a subsequent mitosis. According to the data [6] explanations for increasing poliploidy may lie in hormonal changes. Our results indicating increased rate of polyploidy at untreated hypoplasia accompanied by hormonal disbalance are in good accordance and confirm the rightness of this suggestion. Furthermore, no incidence of polyploidy was observed by us after treating the patient and normalization of hormonal balance.

The acrocentric satellite stalks contain the genes coding for 18s and 28s ribosomal cistrons and represent the Nucleolus Organizing Regions (NORs). Variations in the length and size of satellite stalks and the tendency to enter associations are associated with condensation-decondensation of the region and, hence, chromosome functioning [4, 5].

In this respect we evaluated Ag-staining patterns and chromosome associations in Ag-banded metaphases in the studied person before and after treatment (Fig. 1, 2).

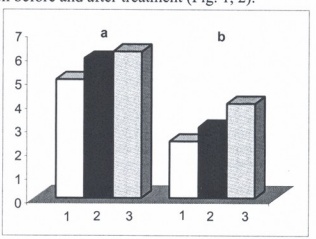

Fig 1. a-number of Ag-positive NOR per cell; b-number of large Ag-positive bands per cell; 1-before treatment; 2-after hormon-therapy with levothyroxin; 3-control

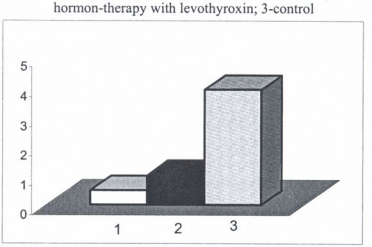

Fig 2. The incidence of Ag-stained acrocentric chromosome associations per cell.
1-index for the patient with thyroid hypoplasia before treatment; 2-index for the patient with thyroid hypoplasia after treatment; 3-mean index for control group.

Decreased content of Ag-positive acrocentrics was found at hypoplasia in both cases -before and after treatment (5.02±0.31 and 5.56±0.33 per cell respectively) as compared with control values (6.33±0.13) that indicates decline of transcriptional activity of nucleolar organizing regions (Fig. 1a). We also registered distribution of large Ag-bands (chromatid-wide or larger in chromosome groups). As it was expected lower

incidence of large Ag-bands were revealed in the studied person 2.52±0.22 before and 3.08±0.24 after treatment (in control – 3.81±0.10) (Fig. 1b).

The evaluation of silver-staining of NORs revealed a positive correlation between staining resolutions of acrocentrics of involved or noninvolved in associations – only Ag-positive chromosomes made associations. The activity of association formation was significantly decreased prior the course of treatment – 0.54±0.1 associations per cell (0.92±0.01 in control) (Fig. 2). After treatment an average number of associations per cell even exceeded the corresponding index for healthy individuals – 1.04±0.14. DG type of associations was most common in all cases than DD or GG types. Chromosomes of D group were more active to enter associations than G chromosomes.

Thus, the frequency of chromosome disorders including a rare form of polyploidy (with fragmented chromosomes) increases, but the transcriptional activity of ribosomal cistrons significantly decreases in the patient suffering from hypoplasia (manifested in low incidence and intensity of silver-staining and chromosome associations). However, after recovery (passing the three-month course of hormonal-therapy), indices of chromosome functional instability start normalizing.

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ქრომოსომათა ფუნქციური მახასიათებლები თიროიდული ჰიპოპლაზიით განპირობებული ჰიპოთირეოიდიზმის დროს

ზოსიძე ნ., კოპლატაძე კ.

გენეტიკის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

ბიოლოგიის კათედრა, ბათუმის სახელმწიფო უნივერსიტეტი

(მიღებულია 28.07.2003)

რეზიუმე

შესწავლილია ქრომოსომათა სტაბილურობა და რიბოსომული ცისტრონების ტრანსკრიპციული აქტივობა ჰიპოპლაზური თირეოიდიტით დაავადებულ ინდივიდში მკურნალობამდე და ლევოთიროქსინით ჰორმონალური მკურნალობის 3-თვიანი კურსის გავლის შემდეგ. თავდაპირველად აღინიშნებოდა ქრომოსომული დარღვევების მაღალი სიხშირე, ფრაგმენტირებული ქრომოსომების შემცველი პოლიპლოიდური უჯრედები და ტრანსკრიპციული აქტივობის დაქვეითება. დაავადების კლინიკური სურათისა და ბიოქიმიური მაჩვენებლების ნორმალიზაციასთან ერთად მოხდა ციტოგენეტიკური მახასიათებლების კორექტირება და დაახლოვება საკონტროლო მაჩვენებლებთან.

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Genetics

THE STUDY OF GENETIC EFFECT OF PESTICIDES IN THE SOYBEAN (*GLYCINE MAX (L) MERR*).

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Abstract

Two pesticides (ridomil, khomecyn) applied in agriculture of Georgia have been studied on their genetic activity in the soybean (*Glycine max (L) Merr.*) by a specially devised test-system. Ridomil was more effective to cause somatic mosaicism and direct mutations than Khomecyn. Both preparations more frequently induced direct mutations than reversions. Moreover, the studied agents didn't differ from each other by their efficiency to induce reservations.

Key words: soybean, pesticide, mutation, somatic mosaicism.

Introduction

Among the negative consequences of scientific-technical progress accumulation and circulation of xenobiotics in the environment should be mentioned. In most cases, besides the toxical activity, chemical pollutants reveal genetic activity as well. They affect the hereditary information of living systems and therefore, the genetic apparatus that has been forming in the progress of evolution is in danger of changing [6].

The pesticides rank first among the pollutants. The main goal of modern applied genetics is to identify the genetically active xenobiotics polluting environment, to study their effect on genetic apparatus, to find protective ways for people and other living organisms in order to defend their hereditary material from harmful influence [2, 3, 4].

Material and Methods

The special line of soybean L65-1237, created by B. Vig and E. Paddock [5] has been used as an object. This line is suitable as it makes possible to identify the genetic disorders caused in somatic cells by studying the phenotypic effect of spots on leaves of the first generation. The experiments were performed on the seeds of heterozygous $Y_{11}y_{11}$ plants. Here the semidominant Y_{11} gene controls the chlorophyll synthesis. The sprouts of three phenotypic classes appear: green ($Y_{11}Y_{11}$), light-green ($Y_{11}y_{11}$) and yellow ($y_{11}y_{11}$), with the ration of 1:2:1 [4, 5]. The results obtained were analysed by the method which counts the spots on the surface of the first two simples and the third complex - leaves. The analysis of the spots was performed using the microscope MBC-9 with magnification - 10 x 2.

The concentrations (0.02%, 0.05% and 0.1% aquaous solution) of studied pesticides (Ridomil, Khomecyn) chosen after the lethal dose (affecting germination and development of seeds) were defined.

Sustained (two-years old) seeds were used in the experiments. 100 air-dried seeds were treated with appropriate concentrations of the preparations for 24 hrs. After treatment the seeds were washed in running water for 3-4 hrs. The studied and control seeds were sowed in the boxes full of sifted soil and black sand (1:1). The results were statistically evaluated.

Results and Discussion

Table 1 shows the genetic changes registered on green plant leaves ($Y_{11}Y_{11}$). Yellow and light-green spots, that are consequences of gene mutations were induced. The light-green spots appear when only one of two alleles has undergone mutations, but in cells that make yellow spots - both alleles are mutated. Yellow spots are more common than light-green ones. In all cases the "dose-effect" phenomena were observed. Ridomil was genetically far more effective than Khomecyn. On the leaves of light-green plants the simple spots (green and yellow) were appearing due to different kinds of genetic disorders (such as: diminutive mutations, deletions, chromosome nondisjunction, etc.) (Table 2). Double spots (with green and yellow halves) that are results of mitotic crossing-over didn't appear. Compared to Khomecyn, Ridomil induced genetic changes in heterozygotic plants more frequently. The phenomenon of "dose-effect" was observed in all variants.

As a result of pesticides effect on the leaves of homozygous yellow plants ($y_{11}y_{11}$) only light-green spots were observed (Table 3), that were caused by reversion in the studied locus. The both preparations in low concentrations induced equal number of reversions. In the case of affecting with a high concentration (0.1%) only a tendency towards increase of Khomecyn-induced changes were revealed. The both preparations induced direct mutations in higher rates compared to reversions.

In the same test-system the influence of 11 herbicides have been studied on one-year seeds. High levels of genetic activity were revealed in the case of induction by Epidor, Heptatiuram and Keltan. All these preparations induced the mutations causing mitotic crossing-over, direct and reverse mutations as well [1]. I. Zakharov et al., studied the effect of physical factors (γ-irradiation, neutrons) and 8 pesticides on the same test-system of soybeans. 7 of them were found to be genetically active, 4 were very effective (Butiphos, Butilcaptos, Cotoran 2,4D). The neutronal influence, unlike γ-irradiation, revealed mutagenic and recombinogenic activities [6].

Table 1. Genetic changes induced in the leaves of homozygous plants green ($Y_{11}Y_{11}$) by pesticides

compound, concentration %	total number of analysed leaves	total number of spots	average number of spots per leaf		in all
			yellow	light-green	
Ridomil					
0,02	72	72	0,65±0,07	0,35±0,09	1,00±0,08
0,05	100	105	0,81±0,09	0,38±0,09	1,19±0,09
0,1	28	149	4,21±0,25	0,64±0,17	4,85±0,21
Khomecyn					
0,02	30	21	0,45±0,14	0,25±0,15	0,70±0,14
0,05	44	38	0,52±0,14	0,34±0,15	0,86±0,14
0,1	32	76	2,10±0,60	0,28±0,17	2,38±0,38
Control	43	24	0,40±0,07	0,10±0,04	0,50±0,05

Table 2. Genetic changes induced by pesticides in the leaves of heterozygous light-green plants ($Y_{11}Y_{11}$)

compound, concentration %	total number of analysed leaves	total number of spots	average number of spots per leaf			in all
			yellow	light-green	double	
Ridomil						
0,02	80	75	0,60±0,10	0,34±0,09	0	0,94±0,09
0,05	62	89	0,98±0,11	0,45±0,10	0	1,44±0,10
0,1	104	353	2,85±0,11	0,55±0,10	0	3,40±0,08
Khomecyn						
0,02	100	80	0,60±0,15	0,40±0,16	0	0,80±0,14
0,05	58	65	0,67±0,12	0,45±0,13	0	1,12±0,12
0,1	79	204	1,95±0,83	0,63±0,32	0	2,58±0,58
Control	61	41	0,45±0,08	0,16±0,06	0	0,61±0,07

Table 3. Pesticide-induced genetic changes in the leaves of homozygous yellow plants ($Y_{11}Y_{11}$)

compound, concentration %	total number of analysed leaves	total number of light-green spots	total number of spots per leaf
Ridomil			
0,02	25	12	0,48±0,14
0,05	20	11	0,55±0,22
0,1	26	16	0,62±0,19
Khomecyn			
0,02	20	7	0,35±0,15
0,05	30	15	0,50±0,17
0,1	16	13	0,81±0,26
Control	21	8	0,35±0,09

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სოიაზე პესტიციდების გავლენის გენეტიკური ეფექტის შესწავლა (*Glycine max (L) Merr.*)

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გენეტიკის კათედრა, ილ. ჭავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

ზოგადი ბიოლოგიის კათედრა, შ. რუსთაველის სახ. ბათუმის სახელმწიფო უნივერსიტეტი

(მიღებულია 28.07.2003)

რეზიუმე

შესწავლილია საქართველოს სოფლის მეურნეობაში გამოყენებული ორი პესტიციდის (რიდომილი, ხომიცინი) გენეტიკური აქტივობა სოიაში (*Glycine max (L) Merr.*) შემუშავებული ტესტ-სისტემის მეშვეობით. რიდომილი უფრო მაღალი სიხშირით, ვიდრე ხომიცინი იწვევს როგორც სომატური მოზაიციზმის, ასევე პირდაპირი მუტაციების ინდუქციას. ორივე პრეპარატი მაღალი სიხშირით ინდუცირებს პირდაპირ მუტაციას, ვიდრე რევერსიებს. რევერსიების ინდუქციით პრეპარატები არ განსხვავდება.

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UV-RAY INDUCED MITOTIC CROSSING-OVER IN CONDITIONALLY LETHAL RADIOSENSITIVE MUTANTS OF WINE YEASTS

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Abstract

In UV-rays induced 14 conditionally lethal radiosensitive mutants of wine yeasts with damaged mitosis, meiosis and reparation the functional test on allelism has been conducted. The mutations belonged to 12 different loci that were found not to be allelic to cdc28, rad2 and rad9 genes. In five lines the UV-rays induced somatic crossing-over was studied. In one mutant line (tvs35) the increase, but in two mutants (tvs33 and tvs69) the decrease of mitotic crossing-over rate were revealed.

Key words: yeast, UV-rays, sensitive mutation, recombination mitosis, meiosis.

Introduction

Reparation of genetic material is in close connection with the processes of replication and recombination. Reparation and recombination have same stages (such as single-strand breaks, resynthesis of regions in DNA chains, splicing of free ends) directing by common enzymes [4,5]. In that way, mutations in the genes that are involved in reparation and crossing-over have a pleiotropic effect. Interdependence of recombination and reparation is well-studied in yeasts [2, 4]. The genes, that participate not only in the regulation of meiosis, but in reparation and crossing-over have been studied [4, 5].

Material and methods

The conditionally lethal radiosensitive tvs mutants induced in GU-90 strain of wine yeast *Saccharomyces cerevisiae var. vini* have been used in our experiments.

Mitotic crossing-over was studied in the system ade2 – his3 (chromosome XV). The linked genes ade2 and his3 are in trans-state and the distance between the two genes equals 34.7 cM. The tvs genes exist in homozygotic state. While studying mitotic crossing-over we compared our results with those for the T2 genetic line constructed in *s.cerevisiae* (genotype MATaMATα ade2-192 ade Δ45rad2 rad2).

The rad2 gene is known to control the process of excision repair. Two mutated alleles of the ADE 1 gene are complementary and are compound. So, the culture is prototrophic and white-coloured. The line has been constructed in St. Petersburg, at the Nuclear Institute and given to us by Prof. I. Zakharov. To identify the alleles of tvs mutants, the auxotrophic genetic lines carrying the cdc28, rad2 and rad9 genes were applied, that had been delivered to us by Dr. I. Arman (Moscow, the Institute of Molecular Biology). The feeding media have previously been described [1]. For inducing recombinative mitosis and meioses the lamp was used as a source of UV-rays, the amount of radiation received – 20 j/m^2/sec. Exposure to the ultraviolet light was performed under the conditions preventing photoreactivation.

Results and Discussion

67 conditionally lethal radiosensitive tvs mutants with failed reparative mitosis and meiosis and halted at 37^0C have been induced with UV-rays. 10 morphs have been identified according to the periods of thermosensitivity. The functional test on allelism was performed in 14 tvs mutants belonging to different morphs. The rad2, rad9 and cdc28 mutations with well-identified loci were used for identification of the studied loci.

The hybrid cells containing the same mutated alleles (compound) do not develop at 37^0C. The process of sporulation is also blocked at this temperature. The Table 1 represents the results of interallele complementation. As it is obvious from the Table, the genes red2, 9 and cdc28 appeared not to be allelic to the analysed tvs mutants. The tvs33 and tvs51 mutations as well as tvs65 and tvs57 loci were found to be complementary. Thus, the same loci were mutated. The 14 studied mutants were revealed to be extended over 12 different loci.

Fig. 2 shows the curves of surviving. According to their radiosensitivity they were distributed as follows: T-GU90, T-35, T-33, T-69, T-2.

Mitotic crossing-over induced with UV-ray has been studied in 5 genetic lines: T-GU90 (control line), T-33, T-35, T-69 and T-2. Fig. 3 demonstrates the curves of the rate of mitotic crossing-over in correlation with the dose of irradiation. Further increase of the dose causes lowering of these values. The lines T-69 and rad2 were found to be highly radiosensitive to the recombinative activities of uv-rays. Our indices for rad2 corresponds to the published data [2]. In rad2 and T-69 the rates of recombinant induction come close or even exceed the control values. In rad2 the excision repair is failed and no falling out of dimers occurs [2, 3]. Similar process is thought to be proceeded in T-69 line cells.

Mitotic crossing-over occurs in remarkably lower rate in T-69 and T-33 lines. In both lines the induction of recombinants grows in parallel with uv-rays doses. The maximal index of crossing-over in T-33 corresponds to 0.77% while in T-69 it is equal to 0.18%. According to these data the both lines resemble the rad54 mutation where the minor reparation is reduced. In the rad54 reparation of double-strand breaks is failed [2]. In T-69 and T-33 the enzymes, that splice the broken chains of the DNA, involved in crossing-over are damaged.

12																	
33	-																
34	-	-															
35	-	-	-														
51	-	+	-	-													
57	-	-	-	-	-												
58	-	-	-	-	-	-											
62	-	-	-	-	-	-	-										
65	-	-	-	-	-	+	-	-									
69	-	-	-	-	-	-	-	-	-								
73	-	-		-	-	-	-	-	-	-							
77	-	-	-	-	-	-	-	-	-	-	-						
82	-	-	-	-	-	-	-	-	-	-	-	-					
94	-	-	-	-	-	-	-	-	-	-	-	-	-				
rad2	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
rad9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
cdc28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
mutation	12	33	34	35	51	57	58	62	65	69	73	77	82	94	rad2	rad9	cdc 28
lokus	tvs1	tvs2	tvs3	tvs4	tvs2	tvs5	tvs6	tvs7	tvs5	tvs8	tvs9	tvs10	tvs11	tvs12	rad2	rad9	cdc 28

Fig. 1 Functional test on allelism (+allelic, –nonallelic mutation)

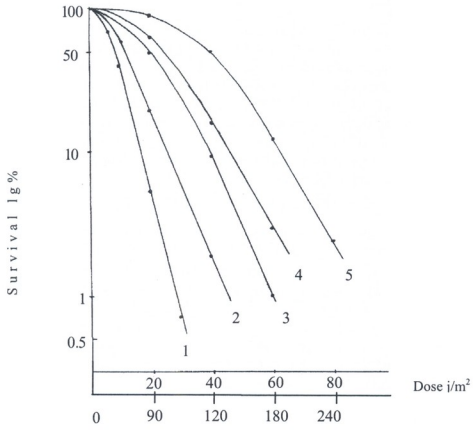

Fig. 2. Dependence of survival on UV-doses
1) T-2. 2) T-69. 3) T-33. 4) T-35. 5) Control TGU-90.

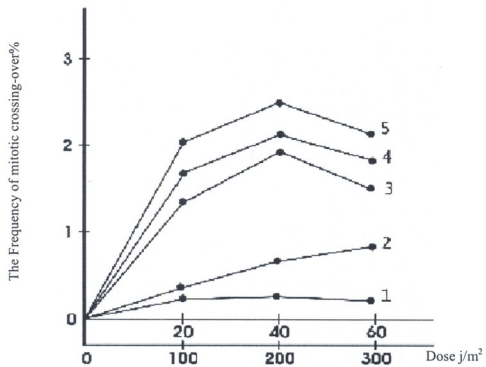

Fig. 3. The Frequency of mitotic Crossing-over in correlation with the dose of uv-rays.
1) T-33. 2) T-69. 3) Control TGU-90. 4) T- 35. 5) T- 2.

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ღვინის საფუარის პიროგითლეტალური რადიომგრძნობიარე მუტანტებში უი-სხივებით ინდუცირებული მიტოზური კროსინგოვერი

ზარნაძე თ., სადაგიშვილი თ., შათირიშვილი ა.

გენეტიკის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტის

(მიღებულია 28.07.2003)

რეზიუმე

უი–სხივებით ინდუცირებულ 14 პიროგითლეტალურ რადიომგრძნობიარე მუტანტში (დარღვეულია: მიტოზი, მეიოზი და რეპარაცია) ჩატარებულია ფუნქციონალური ტესტი ალელიზმზე. მუტაციები 12 განსხვავებულ ლოკუსს მიეკუთვნება, რომლებიც არ აღმოჩნდა ალელური cdc28, rad2 და rad9-ისა. ხუთ ხაზში შესწავლილია უი-სხივებით ინდუცირებული მიტოზური კროსინგოვერი. ერთ მუტანტში (tvs35) მიტოზური კროსინგოვერი გაზრდილია, ხოლო ორში (tvs33 და tvs69) - დაქვეითებულია

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THE STUDY OF ANTIMUTAGENIC ACTIVITY OF VITAMIN COMPLEX "UNDEVIT" IN CASE OF MUTATIONS, INDUCED BY NITRIC FERTILIZERS IN ALBINO MICE

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Abstract

It has been shown that bringing ammonia sultpetre and carbamide (1/2-, 1/5 Ld_{50} dose) in albino mice increases chromosomal aberrations, genome mutations and pathologic mitoses. Vitamins complex "Undevit" revealed antimutagenic and anticytotoxic action. The frequency of induced mutations and pathologic mitoses has been reduced by 2,5.

Key words: ammonia sultpetre, carbamide, "Undevit", vitamins complex, mutagene, antimutagene.

Introduction

Development of chemical enterprise and synthetical chemistry greatly induces chemical mixtures, that we haven't met in nature before. Majority of these compounds have the destructive influence on living organisms and on human beings [8].

Science-technical progress provoked accumulation of "foreign" chemical substances in surroundings, the influence of which has been subordinated by biosphere. The main problem of protecting environment by mutagene is that, the majority of newly produced mutations have negative influence on organism's activity. The negative results of damaging the genetic apparatus of embryonic cells are expressed in populations by increasing mutational load.

Clinical and experimental researches showed, that many biological and chemical factors are characterized by mutagenic action [5].

So, as mentioned above, expression and usage of materials having antimutagenic nature is very important for protecting genetic apparatus of organism from negative chemical factors. Scientific data showed, that some natural and synthetic chemicals are able to reduce mutation level. Antimutagenes were found also among vitamins.

The goal of our research was to determine the protective activity of vitamin complex "Undevit".

Materials and methods

Experiments have been carried out on grown albino mice. Nitric fertilizers, ammonia sultpetre (NH_4NO_3) and carbamide ($CO(NH_2)_2$), in different concentrations (1/2-, and 1/5 Ld_{50}) were used as mutagens. Vitamin complex "Undevit" (therapeutic dose) has been used as antimutagen.

Bringing materials in animals were done perorally by zonde. "Undevit" was brought according to the methods of Ford and Wollam [3] during 5 days preliminary for prophylactic purposes, but on 6th day with mutagenes. On 7th day, the chromosome slides have been prepared from bone marrow cells of animals.

The analyses of genetic disorders have been observed under the light microscopy.

Results and discussion

As stated, that bringing ammonia sultpetre and carbamide in animals induced increase of frequency of chromosomal aberrations (chromosome fragmentation, lysis), genomic mutations (tryproidy and tetraploidy), and pathological mitoses (k-mitoses; hollow metaphases) ($p<0,001$).

In case of bringing sultpetre of dose ½ Ld_{50}, the frequency of chromosome structural aberrations reached 5,84%; pathological mitoses – 21,4%; genome mutations – 2,84%; and hollow interphase nuclei – 4,5% (in control corresponding: 1,0%; 0,2%; 3,7% and 1,1%) (Table).

By influence of "Undevit", violation of chromosome structure induced by ammonia sultpetre (1/2 Ld_{50} dose), (genome mutation, pathological mitoses and hollow interphase nuclei) were reduced to: 2,1%; 1,22%; 8,9%; and 2,0%.

The similar results were reached after the treatment by carbamide. Its bringing in animals (1/2 Ld_{50} dose) induces increase of frequency ($p<0,001$) of violation of chromosome structure (4,51%), genome mutations (2,75%), pathologic mitoses (15,5%), and hollow interphase nuclei (3,0%). By the influence of vitamin complex "Undevit" these indices reduce 2.5 times.

1/5 Ld_{50} concentrations of nitric fertilizers have been investigated in the next series of experiments. The dose–depended effect has been revealed in case of both compounds, i.e. the frequency of genetic alterations, has been decreased as a result of dose reduction. Vitamin complex "Undevit" reduces these violation 2.5 times.

So we stated, that nitric fertilizers – ammonia sultpetre and carbamide are characterized by mutagenic action. They increase the frequency of violation of pathological mitoses and hollow interphase nuclei ($p<0,001$); it seems, that they are characterized by cytotoxical action. By influence of "Undevit" frequency of aberrations have been reduced 2.5 times.

Table. The study on antimutagenic activity of vitamin complex "UNDEVIT" in case of mutations, induced by nitric fertilizers in albino mice.

Variants	The amount of Animals	The amount of metaphases	Dose (mg/kg)	Pathologic mitoses (%)	Chromosome aberrations (%)	Genomic mutation (%)	Hollow interphase nuclei (%)	p
Ammonium nitrate (1/2 Ld_{50})	5	419	175	21,4 ± 2,004	5,96 ± 1,16	2,84 ± 0,81	4,5 ± 0,18	<0,001
Ammonium nitrate + + "Undevit"	5	500	175+7	8,9 ± 1,62	2,1 ± 0,32	1,22 ± 0,24	2,0 ± 0,35	
Ammonium nitrate (1/5 Ld_{50})	4	400	70	15,3 ± 1,79	4,06 ± 0,98	1,67 ± 0,64	2,6 ± 0,29	<0,001
Ammonium nitrate+ + "Undevit"	5	500	70+7	6,1 ± 1,42	1,96 ± 0,38	0,6 ± 0,9	1,5 ± 0,29	
Carbamide (1/2 Ld_{50})	5	400	7103	15,5 ± 1,8	4,51 ± 1,04	2,75 ±1,01	3,0 ± 0,31	<0,001
Carbamide + + "Undevit"	4	400	7103+7	6,3 ± 1,48	2,5 ± 0,61	1,41 ± 0,35	1,6 ± 0,39	
Carbamide (1/5 Ld_{50})	4	400	2841	12,5 ± 1,65	3,1 ± 0,87	2,4 ± 0,51	1,9 ± 0,25	<0,001
Carbamide + + "Undevit"	4	400	2841+7	5,2 ± 1,23	1,4 ± 0,35	0,4 ± 0,009	1,2 ± 0,29	
Control	5	500	-	3,7 ± 0,6	0,8 ± 0,39	0,2 ± 0,19	1,1 ± 0,32	-

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თეთრ თაგვებში ვიტამინების კომპლექსის "უნდევიტის" ანტიმუტაგენური მოქმედების შესწავლა ახოტოვანი სასუქებით მუტაციების დროს

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(მიღებულია 28.07.2003)

რეზიუმე

დადგენილია, რომ თეთრ თაგვებში ამონიუმის გვარჯილას და შარდოვანას (1/2 – და 1/5 LD_{50} დოზა) შეყვანა იწვევს ქრომოსომული აბერაციების, გენომური მუტაციების, პათოლოგიური მიტოზების და ღრუიანი ინტერფაზური ბირთვების სიხშირის სარწმუნო მომატებას.

ვიტამინთა კომპლექსმა "უნდევიტმა" გამოავლინა ანტიმუტაგენური და ანტიციტოტოქსიკური მოქმედება. მის ფონზე აღნიშნული სასუქებით ინდუცირებული მუტაციების, პათოლოგიური მიტოზების და ღრუიანი ინტერფაზური ბირთვების სიხშირე საშუალოდ 2,5-ჯერ შემცირდა.

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Immunology

ABERRANT EXPRESSION OF Fcγ–RECEPTOR (CD64) BY MONOCYTES AND NEUTROPHILS FROM PATIENTS WITH B CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

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Abstract

Targeting of autologous leukaemic cells by CD64-mediated phagocytosis via bispecific antibodies is a promising immunoterapeutic approach for B cell chronic lymphocytic leukaemia (B-CLL). In the present study we examined expression of CD64 on peripheral blood monocytes and neutrophils expressed by percentages as well as a relative density of the expression with the use of flow cytometry. Expression of CD64 on both subsets of blood phagocytes appeared to be aberrant: significant increase in the number of CD64+ neutrophils was detected whilst the numbers of CD64+ monocytes was dramatically decreased. This may reflect a "net effect" of cytokine action.

Key words: B cell chronic lymphocytic leukaemia (B-CLL), CD64, neutrophils, monocytes.

Introduction

B cell chronic lymphocytic leukaemia (B-CLL) is characterized by the accumulation of mature neoplastic B lymphocytes with CD5+ CD19+ phenotype. B-CLL cells are arrested in the G_0/G_1 phases of the cell cycle and neither proliferate nor undergo apoptosis [1, 2].

Bispecific antibodies are designed to recruit monocytes and neutriphils among other types of effector cells. Since a destruction of leukaemic cells can be achieved through Fcγ –Receptor (FcγR) mediated phagocytosis, usage of bispecific antibodies CD64/CD19 directed against FcγRI+ cells and CD19+ B cells is a progressive approach to the treatment of B-CLL [3, 4]. CD64 is a 72 kDa glycoprotein and is expressed on $CD34^+$ myeloid precursors, monocytes, macrophages, dendritic cells and activated neutrophils [5-7]. The purpose of this study was to examine the mode of expression of CD64 on neutrophils and monocytes from B-CLL patients.

Materials and methods

Peripheral blood mononuclear cells (PBMC) from 15 B-CLL patients and 12 age-matched healthy donors were separated on Ficoll-Hypaque (1,077g/l) gradient (Sigma) or after the application of the lysing solution (Gibco). Expression of CD64 marker was measured following the standard immunophenotyping technique. The following monoclonal antibodies were used: FITC~anti Human CD64 and FITC~mouse IgG1 isotype control - (both – Pharmingen). All samples were analysed using a FACScan flow cytometer (Becton&Dickinson). Percentages of cells expressing CD64 marker and mean fluorescent intensity (MFI) of CD64+ cells were measured by gating on monocyte and neutrophil populations in FSC/SSC scatter.

The data was statistically analysed using the Mann-Whitney non-parametrical test. The values given in the table represent average (M) and its standard deviation (SD).

Results and discussion

The results obtained indicate that the percentages of CD64+ monocytes irrespective of the way of their preparation, are significantly decreased, compared to the controls ($p<0.001$), whereas the percentage of CD64+ neutrophils is dramatically increased in patients as compared to normal individuals (Table 1). The picture remains similar for the MFI of monocytes, which is low in B-CLL patients, although the difference is not statistically significant. In case of the neutrophils we observed an increase in a low density CD64 expression as reflected by low MFI.

Table 1. Expression of CD64 marker

Studied subjects	Monocytes				Neutrophils	
	Gradient derived CD64 cells		Lysis derived CD64 cells		Lysis derived CD64 cells	
	%	MFI	%	MFI	%	MFI
Normal controls	75,81±8,14 p<0.001	118,86±67,12	77,51±7,37 p<0.001	137,47±82,03	1,99±0,4 p<0.001	137,46±30,6
B-CLL patients	12,59±11,73	45,13±40,83	14,36±22,21	62,26±60,78	61±5,3	77,8±14,9

The density of CD64 is similar on monocytes of newborns and adults and is normally low on freshly isolated granulocytes. Neutrophils activated *in vitro* by IFNγ, G-CSF or IL-10 have increased density of CD64 [7-9]. On the contrary, IL-4 and IL-13 decrease the level of its expression [7]. Thus, cytokines can affect CD64 expression. A significant increase in the percentages of CD64+ neutrophils *ex vivo* in B-CLL patients can be due to the drastically changed cytokine environment observed through the course of this disease. Increased levels of G-CSF, GM-CSF, IFNγ, IL-10, IL-12 and TGFβ have been documented in serum of B-CLL patients which can affect the expression pattern of CD64 [7-10]. It is indicative that B-CLL neutrophils express low density CD64 which has been previously found only on neutrophils from the patients with sickle cell disease [11]. We have to resume that the same serum cytokine network which led to the overexpression of CD64 on B-CLL neutrophils downregulated the expression of this FcγR on monocytes. In other words there is an aberrant expression of CD64 on both phagocytic populations in blood of B-CLL patients which will influence their functional activity. The search for the exact effector cytokine(s) in both cases is currently underway.

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B-ქრონიკული ლიმფოციტური ლეიკემიით დაავადებული პირების მონოციტებისა და ნეიტროფილების მიერ Fcγ–რეცეპტორის (CD64) ატიპიური ექსპრესია

ახობაძე თ., კაპანაძე თ., ბალოიანი დ., კულიკოვა ნ., ქარდავა ლ., ქართველიშვილი მ. , ჟორდანია ც., გაბუნია ხ., ფორაქიშვილი ნ.

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(მიღებულია 21.07.2003)

რეზიუმე

აუტოლოგიური ლეიკემიური უჯრედების დესტრუქცია შესაძლებელია CD64–ით გაშუალებული ფაგოციტოზის მეშვეობით ბისპეციფიური ანტისხეულების გამოყენებით, რაც იმედისმომცემ იმუნოთერაპიულ მიდგომას წარმოადგენს B-ქრონიკულ ლიმფოციტურ ლეიკემიაში (B-CLL). გამდინარე ციტომეტრის საშუალებით გამოვიკვლიეთ CD64–ის ექსპრესია პერიფერიული სისხლის მონოციტებსა და ნეიტროფილებზე, რაც გამოიხატებოდა მათი პროცენტული მაჩვენებლებითა და აგრეთვე შევისწავლეთ ექსპრესიის შეფარდებითი სიმკვრივეც. CD64–ის ექსპრესია სისხლის ფაგოციტების ორივე ქვეჯგუფისათვის უჩვეულო აღმოჩნდა: დაფიქსირდა CD64+ ნეიტროფილების რიცხვის მნიშვნელოვანი ზრდა და CD64+ მონოციტების მკვეთრი შემცირება. აღნიშნული შესაძლოა ასახავდეს ციტოკინების მოქმედების „ბადის ეფექტს".

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Cytology

COMPARATIVE ANALYSIS OF CARDIOMYOCYTE GROWTH-INHIBITORY FACTOR IN ANIMALS OF DIFFERENT CLASSES

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Abstract

It has been established that low- and high-molecular components of the thermostable proteins of cardiomyocyte growth-inhibitory factor as well as its hydrophilic and hydrophobic components are presented in adult myocardium of different kinds of animals (snail, pigeon, rat, pig). At the same time low- and high-molecular components are identical according to their characteristics in all investigated animals, whereas hydrophilic and hydrophobic components differ from each other. It can be presumed that the following investigation will reveal conformity of low-molecular to hydrophilic, and high-molecular to hydrophobic components, that is important for cardiomyocyte growth-inhibitory factor characteristics.

Key words: Thermostable proteina growth-factor; Cardiomyocytes; Electrophoresis; Hydrophobic interaction chromatography.

Introduction

Among numerous data on growth regulating factors scanty information can be obtained concerning myocardium growth factors [1, 5, 7, 11]. Earlier we have described cardiomyocyte growth-inhibitory factor (CGIF) extracted from myocardium of adult white rat [12]. It has been shown also that the thermostable protein fraction of CGIF consists of two components low- and high-molecular parts [4]. According to our investigation and data of other authors the factor extracted by us is different from those described in the literature [5, 7]. Our CGIF is thermostable and, what is important, has organ-specific and species-nonspecific characteristics [3]. It is well known, that the most of endogeneous growth factors have species-nonspecificity [1], i.e. growth factors obtained from the different organs of different species of animals identically influence on the homologous tissues of other species.

Hence, the goal of the proposed work was comparative analysis of CGIF thermostable proteins in different species of animals.

Materials and methods

Subjects for investigation were: snail, pigeon, rat, and pig. The thermostable protein fractions from myocardium of adult animals were obtained by the method of alcohol precipitation [10]. The native electrophoresis was carried out for separation and comparative analysis of the thermostable protein fractions of CGIF [2]. The method of hydrophobic interaction chromatography was used as well [8]. The

chromatographic analysis was performed on microcolumn high-performance liquid chromatograph Milichrom-4 with automated regulation and UV-detector.

The hydrophilic polymeric sorbent with particle size 10 μm (Hema-Bio-Phenyl-100, Tessek, Praha, Czech Republic) modified by phenyl-groups served as a stationary phase. The mobile phase represented phosphate buffer with ammonium sulfate. Elution was done with mobile phases molarity of those according to $(NH_4)_2SO_4$ varied from 2.0M to 0.0M (up to poor buffer). Detection wavelength was 230 nm.

Results and discussion

Results of electrophoretic investigation are presented on the figure 1: a) snail, b) pigeon, c) rat, d) pig. According to the data analysis the presence of low-molecular subfraction of thermostable proteins of CGIF has been shown in all investigated animals. It appears in all cases as two zones, and molecular weight ranges from 12 to 17 kD (Cytochrom C and Equine myoglobin were used as a marker). High-molecular subfractions of the investigated protein vary within 45-66 kD (Egg albumin and Bovine albumin were used as a marker).

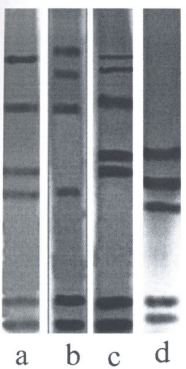

Fig. 1. Electropherogram of thermostable proteins' fractions of CGIF: a) snail, b) pigeon, c) rat, d) pig.

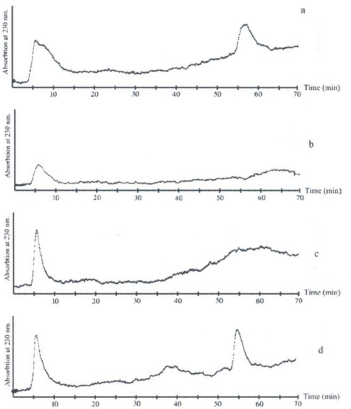

Fig. 2. Chromatogram of thermostable proteins' fractions of CGIF: a) snail, b) pigeon, c) rat, d) pig. (conditions are given in text)

As has been shown in our previous publications, the low-molecular part of the thermostable fraction of CGIF of rat myocardium has mitosis-inhibitory properties, reduces number of active and moderately active nucleoli, and decreases the activation of RNA synthesis in nuclei of cardiomyocytes of newborn rats. As to influence of high-molecular proteins of CGIF on above-mentioned parameters, it was shown by us, that they do not exhibit inhibitory characteristics, but reveal the tissue-specificity [3, 9].

It should be mentioned here about the epidermal growth factor and lymphocytes growth factor as well [1, 6]. The authors presume that endogeneous growth factors consist of the complex of inhibitor, which is low-molecular protein, with high-molecular protein carrier, possessing an immunochemical specificity. Also they emphasize that protein carrier is responsible for organ specificity while protein inhibitor has nonspecific properties.

Our data not only contradict the data of different authors but also confirm and supplement with them. As follows from our results, the low-molecular fractions are found in all investigated animals, moreover

they are identical to each other. We can suppose that species-nonspecificity of CGIF is mediated exactly by this low-molecular component of CGIF.

The results of chromatographic experiments are illustrated on figure 2, which shows that the peak with retention time 5.5 min corresponding to thermostable protein fraction of CGIF is presented in all investigated animals. This is the most hydrophilic component of the protein fraction that according to the retention time coincides with cytochrom C (in identical chromatography conditions). By this method first the most hydrophilic component is released from the proteins precipitated by 2.0M $(NH_4)_2SO_4$ on the chromatographic sorbent. More hydrophobic components are eluted later according to reduction of the concentration of $(NH_4)_2SO_4$.

Analysis of the chromatograms given on the figure 2 allows to suppose that more hydrophobic components of CGIF in different species of animals distinguish from each other both by number of components and the ratio between hydrophilic and hydrophobic components. Namely, the identical hydrophobic components with retention time ~35 min are presented in proteins of myocardium of edible snail and white rat, but only significant elevation of the baseline is observed in the case of pig and negligible rising in the case of pigeon.

Obtained results show that phylogenetically conservative group of thermostable proteins is observed in animals at different stages of their development. The low-molecular and hydrophilic components of these proteins are identical in all classes of investigated animals, but high-molecular and hydrophobic components have different characteristics.

On the basis of proposed data it may be presumed that low-molecular part of thermostable proteins corresponds to hydrophilic proteins, but high-molecular part – to hydrophobic ones.

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ცხოველების სხვადასხვა კლასების კარდიომიოციტური ზრდის მაინჰიბირებელი ფაქტორის შედარებითი ანალიზი

რუსიშვილი ლ., გიორგობიანი ნ., ძიძიგური დ., თუმანიშვილი გ.

განვითარების ბიოლოგიის ლაბორატორია, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 21.07.2003)

რეზიუმე

ცხოველების სხვადასხვა კლასის წარმომადგენლებში (ლოკოკინა, მტრედი, თეთრი ვირთაგვა, ღორი) გამოვლენილია კარდიომიოციტური ზრდის მაინჰიბირებელი ფაქტორის - თერმოსტაბილური ცილების დაბალ- და მაღალმოლეკულური, აგრეთვე ჰიდროფილური და ჰიდროფობული კომპონენტები. ნაჩვენებია, რომ გამოკვლეულ ცხოველებში დაბალმოლეკულური და ჰიდროფილური ცილები თავისი თვისებებით იდენტურია, ხოლო მაღალმოლეკულური და ჰიდროფობული - განსხვავებული.

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Cytology

STUDY OF ENDOGENOUS GROWTH INHIBITORS OF DIFFERENT ORGANS OF WHITE RATS

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Abstract

The effect of termostabile protein factor (TPF) of the organs with different degree of regeneration ability (cardiac, liver and kidneys) of the adult white rats on the proliferative and transcriptive activities of the homologous and non-homologous organs of 7-day old rats has been studied. Received data indicate that TPF of the organs of adult white rats decreases in multiplying tissues RNA's synthesis, inhibits mitotic index and does not reveal tissue-specificity.

Key words: regulation of growth, regeneration, protein factors, transcription.

Introduction

The question of the existence of a factor, limiting growth of organs, and also problems of mutual relation of proliferation and differentiation was proposed by scientists in the last century and is still of interest at present. Different theories were formulated on this question (anti-matrixes, operon and chalone), and each of them had followers and opponents. One of the most widespread and topical for today is the theory of chalone [1] that is based on the regulation of cells' growth by endogenous protein inhibitors. Earlier we described protein factors of adult rats' organs which have tissue specificity [2]. It is well known that various organs are characterized by a different degree of growth and regeneration, for example, cardiomyocyts lose regeneration ability with increase of functional activity, but the liver is highly regenerative organ in the same condition. So, the goal of the given work was investigation of protein growth-inhibitor factors of organs with a different degree of regeneration ability, namely heart, kidneys and liver.

Material and methods

As objects of investigation we studied heart, liver and kidney of white rats. Using a method of alcohol precipitation we received termostable protein fractions - TPFs from the mentioned organs of adult rats [7]. Action of TPFs was investigated *in vivo* on proliferative activity by kolchicin mitotic index (kMI) and *in vitro* on the RNA synthesis by inclusion of the marked precursor in isolated nuclei system in 7-day old rats [3]. The effect of TPF was studied by the following scheme: heart TPF on heart and liver; kidney TPF on kidney and liver; liver TPF on liver and kidney. At least 3 rats have been used for each series of experiment. Data were analyzed using the Student's *t* test.

Results and Discussion

Results of experiments concerning evaluation of kMI after effect of TPF of heart, kidney and liver on homologous and non-homologous organs are shown in Table 1. The table shows that TPF of heart decreases proliferative activity of cardiomyocytes and hepatocytes in about 50-60%. Similar results were received after effect of liver's, and kidney's TPF. It is necessary to pay attention to the fact that in these series of experiments protein factors do not show inherent organ specificity. Earlier we have shown, that tissue specificity of TPF reveals at a level of differentiated organs of adult animals [2], particularly TPF from adult heart decreases RNA synthesis of cardiomiocytes but does not change transcriptional activity of hepatocytes of adult animals.

In Table 2 the influences of investigated fraction on RNA synthesis in isolated nuclei of heart, liver and kidney cells of 7-day old rats are given. Obtained results show that like the mitotic index the intensity of RNA synthesis in nuclei of all types of cells decreases approximately by 40-50%. Thus, on this stage of postnatal development TPF does not reveal tissue specificity.

As it is known, in contrast to striated muscles, all terminal differentiated cardiomyotcytes lose ability to division. All attempts to stimulate division of cardiomyocytes do not give any result [5, 6]. But in response to damage, in less degree in kidney and in more degree in liver of adult animals, rise of proliferation activity is observed, to which precedes activation of RNA synthesis [4].

Our results indicate that TPF of adult heart, kidney and liver of white rats reduce both proliferative and RNA synthesis activity in multiplying tissues. Hence, we can suggest that there is a group of specific proteins (TPF) present in organs with different ability to regeneration that have identical characteristics.

Table 1. The effect of cardiac, kidney and liver TPF on proliferation activity (kMI) of homologous and non-homologous organs.

Cardiac TPF			Liver TPF			Kidney TPF		
	control	trial		control	trial		control	trial
on heart	6.4±0.7	3.8±0.5	on liver	7.2±0.6	4.4 ±0.3	on kidney	9.1±0.9	4.8±0.7
on liver	7.9±0.5	3.7±0.6	on kidney	9.1±0.9	5.8±0.7	on liver	7.7±0.3	3.9±0.2

Table 2. The effect of cardiac, kidney and liver TPF on RNA-synthesys in homologous and non-homologous organs with inclusion of marked precursor.

Cardiac TPF			Liver TPF			Kidney TPF		
	control	trial		control	trial		control	trial
on heart	9697±150	3448±65	on liver	8535±115	4327 ±53	on kidney	7620±34	3660±101
on liver	8535±115	2260±62	on kidney	7620±34	3856±35	on liver	8535±120	5372±105

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თეთრი ვირთაგვას სხვადასხვა ორგანოს ზრდის მაინჰიბირებელი ენდოგენური ფაქტორების შესწავლა

გიორგობიანი ნ., ჩხობაძე მ., რუსიშვილი რ., ძიძიგური დ., თუმანიშვილი გ.

განვითარების ბიოლოგიის ლაბორატორია ციტოლოგიის, ჰისტოლოგიის და განვითარების ბიოლოგიის კათედრა ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 21.07.2003)

რეზიუმე

შესწავლილია ზრდასრული თეთრი ვირთაგვას რეგენერაციის განსხვავებული უნარის მქონე ორგანოების (გულის, თირკმლისა და ღვიძლის) თერმოსტაბილური ციტოვანი ფაქტორების (თცფ) მოქმედება შვიდდღიანი ვირთაგვას სხვადასხვა ორგანოების პროლიფერაციულ და ტრანსკრიპციულ აქტიურობებზე. მიღებული შედეგები მიუთითებს, რომ გამრავლებად ქსოვილებში თცფ თრგუნავენ მიტოზურ ინდექსს, აქვეითებენ rრნმ-ს სინთეზს და არ ამჟღავნებენ ქსოვილსპეციფიკურობას.

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Cytology

THE PECULIARITIES OF THE DIGESTIVE GLAND HISTOARCHITECTONICS IN THE SNAIL *HELIX LUCORUM*

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ABSTRACT

It has been shown that the unknown structures, so called "pink cells", revealed recently within the digestive gland tissue of the snail *Helix lucorum,* represent the cell population characteristic for this species in general. These pink cells have asymmetrically disposed spherical nuclei and acidophilic cytoplasm. Their number in the liver tissue decreases with aging and their revealing carries a seasonal character.

Key words: *Helix lucorum,* digestive gland, cell, regeneration.

Introduction

It has been shown that the "liver" of *Helix lucorum,* like that of rodents, for example, also has the ability for reparative growth. In particular, the peak of transcriptional activity at the 9th hour after partial hepatectomy indicates the occurrence of reparative regeneration in the cells of residual tissue. The appearance of the first mitoses is directly related to the genes reprogramming on the first stages of reparative growth. Herewith the unknown population of so called "pink cells" has been revealed at the estimation of the snail "liver" histoarchitectonics. Based on the fact that the "pink cells" disappear before arising of the first mitoses and that their amount prevails within the forming "liver" tissue, we supposed that these cell-like structures also participate in tissue repair after the hepatectomy. As we found no information on them in available literature, so we decided to investigate their nature and functions in the "liver" tissue.

Materials and methods

Gastropod snails *(Helix lucorum)* were caught in Tbilisi, in the ravine environment of the river Vera. Snails of different age (with body weight accordingly 17 g, 3 g and 500 mg) were used in our experiments. The "liver" was cut off and fixed immediately in Karnua solution (alcohol, chloroform and acetic acid 6:3:1). The small portions of the liver tissue were embedded in paraffin moulds. The light microscopic analysis has been performed on thin sections of about 5 μm stained by means of different methods (Feulgen's reaction, Schiff-periodic acid reaction, Methylene Blue, Light green, Amido Black 10B, Hematoxylin, Eosin) [1]. The "liver" tissue smears were studied as well.

Results and discussion

For present only four types of cells (digestive, calcium, excretory and undifferentiated cells) have been generally described within the digestive gland of terrestrial gastropods [2]. As we have already mentioned the cell-like structures with a pink "cytoplasm" and a small sized "nuclei" attracted our attention in the "liver" histoarchitectonics (Fig. 1). To establish if the dark region stained with hematoxylin within these "pink cells", corresponds to the nuclear material, Feulgen's specific staining for DNA has been used. The purple coloration typical for this reaction has been seen exactly in above-mentioned region. The rest of cell entity was stained with a specific cytoplasmic stains – eosin in one case and light green in other. Thus, our assumption that these liver-specific cell-like structures can be referred to real cells has been confirmed.

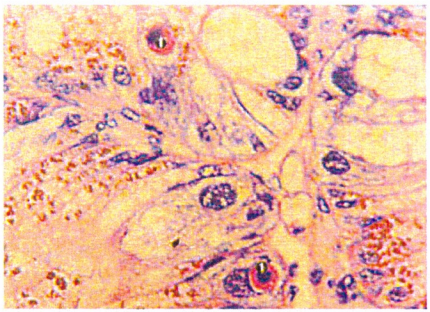

Fig.1. The "liver" tissue stained with Hematoxylin-Eosin (10x40): 1 – the "pink cell"

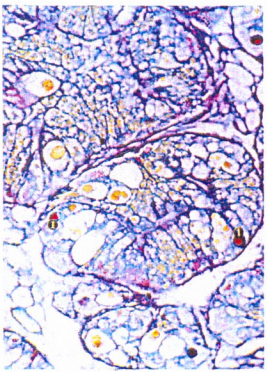

Fig. 2. The "liver" tissue stained with Amido Black (10x20): 1-the "pink cell"

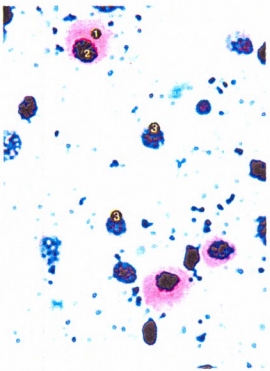

Fig. 3.The "liver" tissue smear stained with Methylene Blue and Eosin (10x40):, 1-the "pink cell" cytoplasm, 2 -the "pink cell" nucleus, 3-nuclei of the other "liver" cells

As one of the main "liver" functions is to store glycogen, it seemed interesting to find out whether "pink cells" take part in this process. For that Schiff-periodic acid reaction – the specific staining for polysaccharides - has been used. The pink coloration which occurred only within the "pink cells" cytoplasm, indicates that these cells are the storage of sugar-containing substances in the "liver" lobules.

A very interesting picture has been obtained after staining the "liver" tissue sections with Amido Black. Against a greenish-bluish background of the "liver" tissue the "pink cells" of dark red colour are clearly visible (Fig. 2), thus, we propose this staining to be used as a differential staining method for the "pink cells" in the future investigations.

A noteworthy peculiarity has been shown during the study of the "liver" tissue smears. As it's shown in the figure only the "pink cells" preserve their cell entirety on the smears (Fig.3). It might be explained by their weak connections within the "liver" structure, so that they can be separated easily from the "liver" tissue without a cytoplasm damage.

During our experiments it has been shown that a quantitative composition of the "pink cells" within the "liver" tissue depends on the animal's age. In particular, along with aging a number of "pink cells" gradually decreases (7% in 17 g snails, 3% in 3 g and 1% in 500 mg snails). Furthermore, "pink cells" are displayed in "liver" tissue in Spring (April/May) after the hibernation and completely disappear at the end of Summer.

In the end we can conclude that the unknown cell-like structures, discovered in the "liver" of the snail *Helix lucorum*, with an asymmetrically disposed nucleus and acidophilic cytoplasm really represent cells. A correlation exists between molluscan age and number of the "pink cells". Their appearance within the liver tissue is of seasonal character.

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HELIX LUCORUM-ის საჭმლის მომნელებელი ჯირკვლის ჰისტოარქიტექტონიკის ზოგიერთი თავისებურების გამოკვლევა

ჭილაძე ნ., მოდებაძე ი., თუმანიშვილი თ., კვინტრაძე თ., ძიძიგური დ.

ციტოლოგიის, ჰისტოლოგიისა და განვითარების ბიოლოგიის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 21.07.2003)

რეზიუმე

ჩატარებული გამოკვლევებით დადგინდა, რომ *Helix lucorum*-ის "ღვიძლის" ქსოვილში ჩვენს მიერ ადრე აღნიშნული უცნობი სტრუქტურები ე. წ. "ვარდისფერი უჯრედები" წარმოადგენენ ამ სახეობისათვის დამახასიათებელ უჯრედების პოპულაციას. მათ გააჩნიათ მკვეთრად გამოხატული სფერული ფორმა, ასიმეტრიულად განავსებული ბირთვი და აციდოფილური ციტოპლაზმა. "ღვიძლის" ქსოვილში "ვარდისფერი უჯრედების" რაოდენობა მცირდება მოლუსკის ასაკის მატებასთან ერთად და მათი გამოვლენა სეზონურ ხასიათს ატარებს.

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Cytology

THE STUDY OF THE PECULIARITIES OF FROG LIVER REGENERATION AT THE EARLY STAGE OF REPARATIVE GROWTH

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Abstract

The study presents the peculiarities of frog (Rana ridibunda) liver regeneration at the early stage of reparative growth. It has been shown that the process of reprogramming of growth controlling genes in hepatocytes stimulated for proliferation is realized similarly both in rodents and filogenetically distant animals (such as frogs). In particular, like in rodents, in tailless amphibians the hepatocytes transcriptional activity peak reveals at the 6th hour after partial hepatectomy and the early mitoses shows up within 32-52 hours after operation. The appearance of early mitoses in regenerating liver of different animals, which is related to the first peak of transcriptional activity, may be considered as general phenomenon for hepatocytes stimulated for proliferation.

Key words: hepatectomy, reparative growth, frog's liver, mitosis, early response genes.

Introduction

At present, the process of liver reparative growth has been intensively studied in rodents [2,4]. It is well known that at the early stage of liver reparative growth the reprogramming of growth controlling genes takes place [2,3]. A little is known about the liver regeneration at the early stage of reparative growth in filogenetically distant organisms. That's why as a research object we chose a representative of tailless amphibians – Rana ridibunda characterised by low regenerative ability of liver [5,6].

According to this, the goal of our present work was to establish the general principles of trigger mechanisms of liver regeneration in filogenetically distant organisms, especially to study the peculiarities of growth controlling genes expression in animals with low regenerative ability of liver.

Materials and Methods

The adult frogs (Rana Ridibunda) were used in the experiments. Partial hepatectomy (1/3 of liver tissue was removed) was performed in the morning hours. To evaluate the changes in hepatocytes transcriptional activity the liver tissue was taken once in an hour during the day. The isolation of nuclei was carried out by the methods described earlier [1]. Transcriptional activity was evaluated by the intensity of C^{14}-UTF uptake [1]. Mitotic index was determined on the 5 μm sections stained with hematoxylin – eosin. Statistical analysis was performed by Student's test.

Results and Discussions

To evaluate the transcriptional activity of amphibian regenerating hepatocytes at the early stage of reparative growth, first, we have studied RNA-polymerase activity of intact hepatocytes during the day, through which the circadian changes of transcriptional activity have been revealed [Fig 1.a.]. As mentioned above, the regenerative growth in rodent's (white rat) liver starts with genes' reprogramming. Within the first five minutes after operation the amount of immediate early genes (c-fos, c-jun, c-myc) products increases. Products of these genes activate the delayed early genes, which transcriptional activity peak reveals at the 6th hour post operation [3,4]. To establish whether the genes reprogramming is realised in similar manner in frog's liver, the experimental animals were hepatectomized. We have studied changes in transcriptional activity within the first ten hours after partial hepatectomy. The analysis of obtained data revealed the transcriptional activity peak in frog's liver at the 6th hour after operation, just like in white rats [Fig. 1. b.].

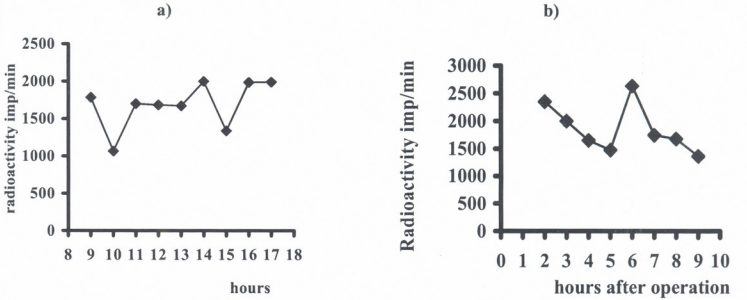

Fig. 1. The changes of RNA-synthesis activity in frog liver cell nuclei in norm (a) and after partial hepatectomy (b)

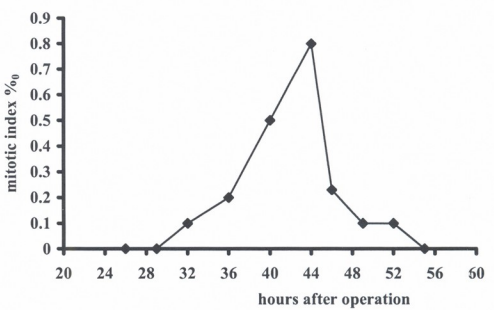

Fig. 2. The changes of mitotic activity in frog liver tissue after partial hepatectomy

According to literature data the first peak of transcriptional activity in rat hepatocytes in response to the proliferative stimulus is directly related to the appearance of mitoses at the 24th hour after operation [1]. Based on this, we suggested the possibility of early mitoses presence in frog's regenerating liver cells. To check our suggestion we determined mitotic index of hepatocytes during four days after partial hepatectomy. In our experiments the first mitoses in liver tissue was revealed at the 32nd hour after operation. The mitotic activity revealed within 32-52 hours' interval reaching maximum at the 44th hour after operation.

Based on the obtained results we suggest, that the process of reprogramming of growth controlling genes in hepatocytes stimulated for proliferation is realized similarly both in rodents and filogenetically distant animals (such frog -Rana ridibunda). At the early stage of reparative growth the appearance of early mitoses related to the first peak of transcriptional activity is characterised also for animals with low regenerative ability of liver.

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ადგენითი ზრდის საწყის ეტაპზე ბაჭაჭის ღვიძლის რეგენერაციის თავისებურებების შესწავლა

გურუშიძე მ., აბრამიძე თ., კლიმიაშვილი თ., კახიძე ი., ძიძიგური დ.

ციტოლოგიის, ჰისტოლოგიის და განვითარების ბიოლოგიის კათედრა, განვითარების ბიოლოგიის ლაბორატორია, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 21.07.2003)

რეზუმე

დადგენილია ბაყაყის (Rana ridibunda) ღვიძლის რეპარაციული რეგენერაციის თავისებურებები აღდგენითი ზრდის საწყის ეტაპზე. პროლიფერაციისადმი სტიმულირებულ ღვიძლის უჯრედებში ზრდის მაკონტროლირებელი გენების რეპროგრამირება მსგავსი სქემით ხორციელდება მღრღნელებისაგან ფილოგენეზურად დაშორებულ უკუდო ამფიბიების წარმომადგენელში. კერძოდ, ბაყაყის ჰეპატოციტების ბირთვების ტრანსკრიპციული აქტიურობა პიკს, მღრღნელების მსგავსად, ნაწილობრივი ჰეპატექტომიიდან მე-6 საათზე აღწევს. ნაჩვენებია, აგრეთვე, ღვიძლის ქსოვილში ადრეული მიტოზების აღმოცენება (ოპერაციიდან 32-52სთ). სხვადასხვა ორგანიზმების რეგენერირებადი ღვიძლის ქსოვილში ტრანსკრიპციული აქტიურობის პირველ პიკთან დაკავშირებული ადრეული მიტოზების აღმოცენება პროლიფერაციისადმი სტიმულირებული ღვიძლის უჯრედებისათვის დამახასიათებელ კანონზომიერებად შეიძლება ჩაითვალოს.

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Cytology

INFLUENCE OF SOME BENZODIAZEPINES ON THE TRANSCRIPTIONAL ACTIVITY OF LUNG CELLS OF THE EXPERIMENTAL ANIMALS

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Abstract

The influence of some of benzodiazepines on the genes expression of lung tissue cells of the experimental animals has been studied. It was established, that the using of halothane as anesthetic results in the inhibition of the transcription process of lung tissue cells of white rats. It has been shown that the inhibition effect of RNA synthesis stipulated by narcosis is neglected in the case of premedication via midazolam and diazepam.

Key words: midazolam, diazepam, halothane, genes expression, lung tissue.

Introduction

Premedication is a necessary component of anesthesia in orthodontic surgery in childhood because of its main function: it decreases stress, providing the sedative effect and minimizing post-operative neurovegetative side effects [1]. To accomplish all these in children drugs of benzodiazepine group – midazolam and diazepam - are used, instead of drugs of narcotic origin. Their antihypoxic effect is also noted in children. It is very important to use them in children with palate and lip clift, who have phonic hypoxia. That's why we decided to study the effects of these drugs on gene expression in pulmonary tissue in experimental animals.

Matherials and methods

Adult white 40 rats (120-140g) were used in experiments. The transcriptional activity of isolated nuclei was evaluated by the intensity of insertion of ^{14}C-UTF [2]. To reduce post-operative hypoxia drugs midazolam and prednizolon were selected. For anesthesia we used halothane. Experimental animals were

divided into 5 groups: 1- group was used as control, 2 – intact animals, on which pseudo-operation was realized using anesthesia, 3- 30 minutes before anesthesia diazepam (0.2 mg/kg) was injected with anesthesia of halothane and pseudo-operation; 4 – 30 minutes before anesthesia midazolam (0.2 mg/kg) was injected with halothane anesthesia and pseudo-operation. The duration of depressive effect of halothane was studied over 24 hours and over one week.

Results and discussions

It was estimated that halothane inactivates transcription in cells of pulmonary tissue. The transcriptional activity of isolated nuclei is reduced by 21% in comparison to control (Fig. 1). Recently we demonstrated that anesthetic effect of inhibition of RNA-synthesis is diminished in the case of using diazepam in premedication. But the stimulation of RNA synthesis was 30% (Fig. 1). The transcriptional

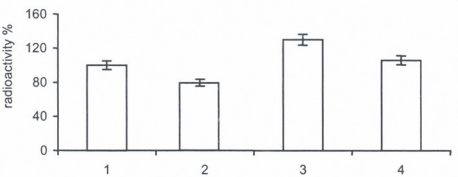

Fig.1. Influence of some benzodiazepines on the transcriptional activity of lung cells of the adult rat

1. Control (Intact animals)
2. Anaesthesia via halothane and false operation
3. Diazepam (0.5hour before operation), anaesthesia via halothane and pseudo- operation
4. Midazolam (0.5hour before operation), anaesthesia via halothane and pseudo- operation

activity of nuclei is also increased by 27% in case of midazolam injection 30 minutes before operation (Fig. 1). Concerning the results, it was interesting to find out the ability of organism to regenerate the initial level of genes' expression in cells without using above mentioned drugs. We found out that in 24 hours after the operation the inhibitory effect of halothane on transcriptional activity of nuclei is decreased (Fig. 2). After 1 week from the operation the RNA-synthesis in above-mentioned cells is stimulated by 34%. Experiment results show that in the critical period for functional activity of lung cells caused by anesthesia and operation (when posthypoxic changes develop), positive effects of preaneasthetic medication via diazepam and midazolam occur.

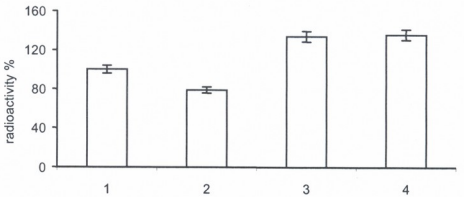

Fig. 2. Influence of halothane on the transcriptional activity of lung cells of the adult rat

1. Control (Intact animals)
2. 1 hour after anaesthesia via halothane and pseudo-operation
3. 24 hours after anaesthesia via halothane and pseudo-operation
4. 1 week after anaesthesia via halothane and pseudo-operation

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ბენზოდიაზეპინის ჯგუფის ზოგიერთი პრეპარატის ზემოქმედება ექსპერიმენტული ცხოველების ფილტვის უჯრედების ტრანსკრიპციულ აქტიურობაზე

ძიძიგური ლ.[1], კაპანაძე გ.[3], მხითარიანი დ.[3], გიორგობიანი მ.[1], ვადაჭკორია ზ.[2]

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[2]ბავშვთა ასაკის სტომატოლოგიის კათედრა, სახისა და ყბის განვითარების თანდაყოლილი მანკების მქონე ბავშვების დისპანსერიზაციის, მკურნალობის და რეაბილიტაციის რესპუბლიკური ცენტრი, თბილისის სახ. სამედიცინო უნივერსიტეტი
[3]ციტოლოგიის, ჰისტოლოგიის და განვითარების ბიოლოგიის კათედრა ივ.ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 21.07.2003)

რეზიუმე

დადგენილ იქნა, რომ ექსპერიმენტულ ცხოველებში ანესთეზიის მიზნით ჰალოტანის გამოყენება იწვევს გენების ექსპრესიის დაორგუნვას მხოლოდ ფილტვის ქსოვილის უჯრედებში. რნმ-ის სინთეზის ნარკოზით განპირობებული ინჰიბირების ეფექტი პრემედიკაციის შემთხვევაში იხსნება დიაზეპამით და მიდაზოლამით. მიღებული შედეგები კარგად კორელირებს კლინიკური გამოკვლევების მონაცემებთან, რომელთა თანახმადაც ამ პრეპარატების გამოყენების შემთხვევაში ყბა-სახის პათოლოგიების მქონე ბავშვებში არ აღინიშნება პოსტოპერაციული გართულებები.

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THE INFLUENCE OF HEPAR COMPOSITUM ON REGENERATIVE GROWTH OF WHITE RAT HEPATOCYTES AFTER PARTIAL HEPATECTOMY IN CONDITIONS OF HORMONAL DISBALANCE

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Abstract

The influence of antohomotoxic drug Hepar compositum on transcriptional activity of hepatocytes of adult white rat was studied in conditions of hormonal disbalance (bilateral adrenalectomy). After intraperitoneal injection of drug the transcriptional activity of hepatocytes' nuclei was increased for 38% compared to control group and had no effects on transcriptional activity of kidney epithelial cells' nuclei. From our results we can conclude that the effects of Hepar compositum is organ-dependent.

Key words: Hepar Compositum, partial hepatectomy, bilateral adrenalectomy, transcription, early response genes.

Introduction

It's known that the products of transcription of immediately early response genes appear within 30 minutes after partial resection of liver in rats. These products represent the transcriptional factors for delayed early response genes and the first peak of their transcriptional activity appears on the 6th hour after stimulus for proliferation [1]. Under conditions of hormonal disbalance (bilateral adrenalectomy) the changes corresponding to early stage of reparative growth are also seen in hepatocytes. After partial resection of liver on forth day of adrenalectomy the above-mentioned peak appears much earlier – on the 3rd hour after the operation [2]. Earlier we demonstrated that antihomotoxic drug, Hepar Compositum has stimulatory effect on transcription of early response genes [3]. In present work we made an attempt to reveal whether this drug keeps the same effect on hepatocytes in condition of hormonal disbalance.

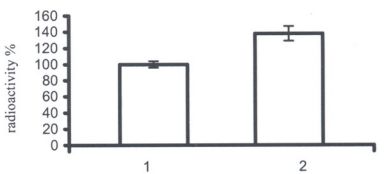
The aim of our investigation was to reveal the effect of Hepar Compositum on regenerative growth of hepatocytes of white rats after partial hepatectomy in conditions of hormonal disbalance.

Materials and methods

Adult white rats (120-140g) were used in experiments. The research was conducted using the experimental models of the partial hepatectomy and bilateral adrenalectomy [4]. Transcriptional activity of hepatocyte nuclei was assessed using methods described earlier [5]. Animals were divided into two groups. The first group was used as control and animals of the second group were injected by 2 mcl of Hepar Compositum. All injections were intraperitoneal.

Results and discussions

We demonstrated that injection of Hepar Compositum had stimulatory effect on hepatocytes' nuclei in conditions of hormonal disbalance – the transcriptional activity increased by 38 % (Fig. 1). The stimulatory effect of Hepar Compositum on hepatocyte nuclei proves once more that antihomotoxic drugs have ability to stimulate the transcriptional processes in cells and thus initiate the early stage of reparative growth. It's well known that the curative effects of antihomotoxic drugs are based on their ability to activate immune system. After administration of drug the so-called "motives" appear – sequences which consist of 9-15 amino acids. This induces production of Th3 lymphocytes, which in turn produce TGF-β. And because TGF-β has the ability to activate genes that control proliferation, the stimulatory effect of Hepar Compositum on early response genes may be of this origin. In contrast, the drug had no effect on renal epithelial cell nuclei in the same conditions (hormonal disbalance) (Fig.2). We can conclude that the effects of antihomotoxic drugs are organ-dependent. Our data is in positive correlation with literature findings – it's estimated that each antihomotoxic drug contains substances, which are derived from target organ [6].



1. Bilateral adrenalectomy (4th day)
2. Bilateral adrenalectomy (4th day) +Hepar Compositum (2 mcl)

Fig. 1. The influence of Hepar Compositum on transcriptional activity of rat's hepatocytes' nuclei in bilateral adrenalectomy (4^{th} day).

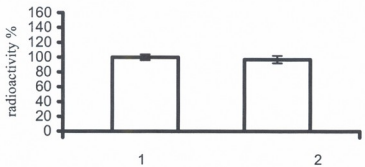


Fig. 2. The influence of Hepar Compositum on transcriptional activity of rat renal epitheliocytes nuclei in bilateral adrenalectomy (4^{th} day).

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Hepar Compositum-ის ზეგავლენა თეთრი ვირთაგვას ჰეპატოციტების ტრანსკრიპციულ აქტიურობაზე ნაწილობრივი ჰეპატექტომიის შემდეგ ჰორმონული დისბალანსის პირობებში

ლუარსაბიშვილი ვლ.[1], ცაგარელი ზ.[1], მეგრელიშვილი გ.[3], თავდიშვილი ე.[2], მაისურაძე ნ.[2]

[1] ა. ნათიშვილის სახელობის ექსპერიმენტული მორფოლოგიის ინსტიტუტი
[2] ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი
[3] კ. ერისთავის სახელობის ექსპერიმენტული და კლინიკური ქირურგიის ეროვნული ცენტრი

(მიღებულია 21.07.2003)

რეზიუმე

შესწავლილია ანტიჰომოტოქსიკური პრეპარატის, Hepar Compositum-ის ზემოქმედება თეთრი ზრდასრული ვირთაგვას ჰეპატოციტების ბირთვების ტრანსკრიპციულ აქტიურობაზე ჰორმონული დისბალანსის პირობებში (ორმხრივი ადრენალექტომია). ნაჩვენებია, რომ Hepar Compositum-ის ინტრაპერიტონიალურად შეყვანის შედეგად ჰეპატოციტების ბირთვების ტრანსკრიპციული აქტიურობა კონტროლთან შედარებით 38%-ით იზრდება. პრეპარატის ინექცია არ ახდენს ზეგავლენას თირკმლების ეპითელიოციტების ბირთვების ტრანსკრიპციულ აქტიურობაზე. მიღებული შედეგებიდან გამომდინარეობს, რომ ანტიჰომოტოქსიკურ პრეპარატს ახასიათებს ორგანოსპეციფიკურობა.

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INFLUENCE OF FLAVONOID FRACTION FROM *SATUREJA HORTENSIS* ON MICROSOMAL OXIDATION

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ABSTRACT

Inhibitory effect of flavonoid fraction from *Satureia hortensis* on arachidonic acid oxidation in rabbit liver microsomes is shown. This flavonoids probably affect enzymatic system of prostaglandins biosynthesis. Antioxidant property of flavonoid fraction was also shown, as it decreased malon dialdehyde concentration in liver microsomes. High inhibitory effect of flavonoids on microsomal oxidation of well-known xenobiotic, dimethilanyline by cytochrome P450 was also studied.

Key words: Phenolic fraction, arachidonic acid, cytochrome P450, malon dialdehyde.

INTRODUCTION

Lately one of the most perspective strategies of biological research is study of herbal bioactive compounds from medical and pharmacological point of view, as negative side effects of some antibiotics and anti-inflammatory drugs were proved [1]. On the other hand positive effect of herbal phenolic compounds on several vital functions of organism was shown, their influence on cytochrome P450 dependent and cyclooxigenase pathways was studied [2, 3]. The purpose of this study was to examine effect of phenolic fraction (flavonoids) from annual plant *Satureja hortensis* (summer savoury) on biological systems. Summer savory has been used for medical approaches for ages, but molecular mechanisms of its flavonoids still remain unknown [4, 5]. We have studied effect of these flavonoids several microsomal oxidative enzyme systems, such as cytochrome P450, cyclooxigenase and lipoxigenase. Arachodonic acid (AA) is mainly used by those enzymes as a substrate [6, 7, 8].

MATERIALS AND METHODS

Phenolic fraction (F) was obtained from *Satureja hortensis*, as previously described [9]. Micrisomal fractions were isolated from the liver of male (2,5-3kg) rabbits by ultracentrifugation [10]. Cytochrome P450 concentration was measured on spectrophotometer [11]. We observed oxidative activity of microsomes by polarographic method at 37^0C [1,10] and peroxidation by measuring changes in malondialdehyde (MDA) concentration [12]. Methanol solution of AA (Sigma) was used (33μM). Other reagents, that were used are: Indometacine and Aspirin (ASP) (10-10μM), NADPH (1,34μM) (Sigma), 1% solution of savory flavonoids (100μl). Gas of CO was used for cytochrome P450-dependent monooxigenation inhibition in microsomal fraction. Saturated solution (100μl) of well-known xenobiotic, dimethilanylin (DMA) was used. Protein

concentration was measured according to Bradford assay [13]. $FeSo_4 \cdot 7H_2O$ (50μM) and also NADPH (0,2μM) was used to initiate peroxidation.

RESULTS AND DISCUSSION

Usage of classical xenobiotic DMA proved normal functioning of cytochrome P450 in microsomes, as NADPH activated oxidation of DMA strongly and CO inhibited the process by 23%. Our phenolic fraction (F) was found to be much stronger inhibitor of DMA oxidation (~60%), than CO. These data show, that F has strong inhibitory ability of cytochrome P450 (Fig. 1).

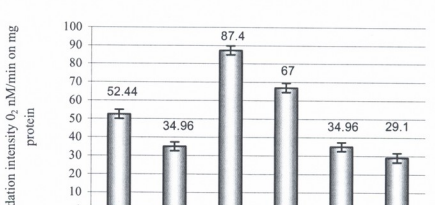

Fig.1. The influence of savory flavonoides and carbone monoxide on oxidation of DMA by cytochrome P450-dependent monoxigenaze.

1) NADPH; 2) NADPH+CO; 3) DMA+NADPH; 4) DMA+NADPH+CO 5) DMA+NADPH+F; 6) DMA+NADPH+F+CO.

We were interested, whether F fraction affected AA oxidation. The results obtained indicated, that CO inhibited this process by ~10%, while F fraction was stronger (by 57%), that reveals unimportant role of P450 in microsomal oxidation of AA. AA was used by some other enzyme system, probably cyclooxigenase. NADPH increased intensity of oxidation more than twice, and CO had no effect on the process. On the other hand we have observed some increase in intensity of oxidation, that might not to be fluctuation. Probably, CO blocked cytochrome P450 and NADPH was used mainly by AA oxidative systems and was not wasted on monooxigenation of other substrates by cytochrome P450, because F fraction inhibited this process rather potently (by 48%). Adding of more CO was ineffective either (Fig. 2).

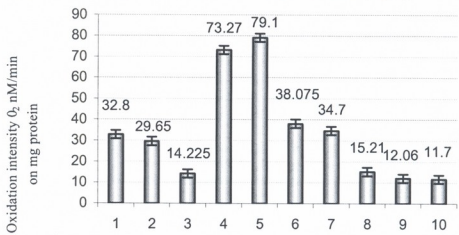

Fig. 2. The influence of savory flavonoids on the oxidation of arachidonic acid in rabbit liver microsomes.

1) AA; 2) AA+CO; 3) AA+F; 4) AA+NDAPH; 5) AA+CO+NADPH; 6) AA+NDAPH+F; 7) AA+CO+NDAPH+F; 8) AA+NDAPH+ASP; 9) AA+ASP; 10)AA+INDOMETACIN

The experiment was held to study influence of Aspirin and Indometacine on AA oxidation as well. Achieved results showed, that both these specific inhibitors of cyclooxigenase strongly inhibited AA oxidation in microsomal suspension, that once more emphasises the fact, that prostaglandin synthesis really occurs in liver microsomes (Fig. 2). These data are valuable itself, as prostaglandins besides their multiple physiological functions, play negative role in inflammation, tumour and some other pathologic processes [6,7,8] and finding of such natural inhibitors, as flavonoids of savory would be considerable advance against pathologies mentioned above.

One more active oxidative process, that occurs in microsomes is lipid peroxidation. It is considered as physiological process, but its increased intensity is also associated with pathology [12]. After 30 minutes incubation levels of peroxidation that were seen for control (K), samples with Fe^{2+}, NADPH and with $Fe^{2+}$and NADPH together are shown in Fig.3. F fraction inhibited peroxidation in each of these samples (Fig.3): in control it inhibited the process by 30%, in microsomes with Fe^{2+} - by 36%, in microsomes with NADPH oxidation was decreased by 55% and in microsomes with both Fe^{2+} and NADPH - by 47%. Due to these data we can conclude, that F fraction from savory is the inhibitor of peroxidation as well.

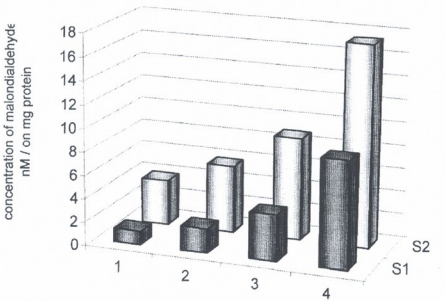

Fig. 3. The influence of savory flavonoids on the intensity of peroxidation
S1 - 1) K+F; 2) K+Fe^{2+}+F; 3) K+NADPH+F; 4)K+NADPH+ Fe^{2+}+Fe;
S2 - 1) K; 2) K+ Fe^{2+}; 3) K+NADPH; 4)K+NADPH+ Fe^{2+}.

We have to resume, that AA is oxidated by microsomal enzymes itself. NADPH increases oxidation. Cytochrome P450 takes rather unimportant part in these processes and the main enzyme system in AA oxidation must be cyclooxigenase, that was inhibited by F fraction. As for the peroxidative processes, the same F fraction inhibits both induced and uninduced peroxidation in rabbit liver microsomes.

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Satureja hortensis -ᲓᲐᲜ ᲒᲐᲛᲝᲛᲧᲝᲤᲘᲚᲘ ᲤᲚᲐᲕᲝᲜᲝᲘᲓᲔᲑᲘᲡ ᲨᲠᲐᲤᲪᲘᲘᲡ ᲒᲐᲕᲚᲔᲜᲐ ᲛᲘᲙᲠᲝᲡᲝᲛᲣᲚ ᲕᲐᲜᲒᲕᲐᲖᲔ

ქუჩუკაშვილი ზ., ავალიანი ნ., დავითაია გ.

ბიოფიზიკის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი,

(მიღებულია 21.07.2003)

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ბოცვრის ღვიძლის მიკროსომულ ფრაქციაში ნაჩვენებია ***Satureja hortensis***-დან მიღებული ფლავონოიდების ფრაქციის მაინჰიბირებელი გავლენა არაქიდონის მჟავის ჟანგვაზე. ინჰიბირება სავარაუდოდ ეხება პროსტაგლანდინების ბიოსინთეზის ფერმენტულ სისტემებს. დადგენილია აგრეთვე ფლავონოიდების ფრაქციის ანტიოქსიდანტური თვისებები, რაც გამოიხატება მიკროსომებში ზეჟანგური ჟანგვის ერთ-ერთი საბოლოო პროდუქტის, მალონის დიალდეჰიდის კონცენტრაციის შემცირებით. გამოვლენილია ფლავონოიდების ძლიერი ინჰიბიტორული ეფექტი მიკროსომული ფერმენტის, ციტოქრომ P450-ის მიერ ცნობილი ქსენობიოტიკის დიმეთილანილინის ჟანგვაზე.

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Biophysics

THE CHANGES OF MITOTIC ACTIVITY AND THE CHOLESTEROL LEVEL UNDER INFLUENCE OF PHENOLIC COMPOUNDS FROM *SATUREJA HORTENSIS* ON ONE-SIDE NEPHRECTOMED RATS

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ABSTRACT

The phenolic fraction F received from summer savory (*Satureja hortensis)* causes the considerable decrease of cholesterol level in rats before and after one-sided nephrectomy, in comparison with control tests. In analogous tests with indomethacin the effect shows only on the second day after operation which should be referred due to its specific and reversible affect. Fraction F, in analogous tests, also causes the considerable increase of mitotic index on the second day after nephrectomy and this effect is higher at postoperative injection of fraction F. At preoperative injection of fraction F this effect is stored and the level is high, whereas at postoperative injection the mitotic index drops to zero (5-th day after a nephrectomy). Analysis of obtained experimental data shows, that fraction F has nonspecific and wider action spectrum in comparison with indomethacin.

Key words: *Satureja hortensis,* phenolic compounds, one-side nephrectomy, cholesterol, mitotic index,

INTRODUCTION

It is known, that the cholesterol plays the important role during vital activity of an organism and breaking its function is the cause of development of many inflammatory pathologies, such as atherosclerosis and hypertension. In particular, dysfunction of microvasculature is associated with development of a sugar diabetes [1]. Mechanisms of atherogenesis include lipoprotein level changes, the changes in composition of lipoproteins influencing on binding of low-density lipoproteins (LDLP) with its receptors, decrease of outcome of LDLP, which are the source of cholesterol [2]. The processes of peroxide oxidation of lipids promoting such changes are also revealed. In particular, it is shown the formation of 8-epi-$PGF_{2\alpha}$ by not enzymatic way. 8-epi-$PGF_{2\alpha}$ is the marker of peroxidative reactions and provides intensifying process of a hypercholesterolemia [3]. On the other hand, in our investigations [4] were shown a presence of high antioxidative and antimicrobial properties of phenolic compounds from *Satureja hortensis,* which have been successfully checked against various processes of a thermal lesions [5].

MATERIALS AND METHODS

The aim of current investigation was to study the influence of fraction F [6] on the regeneration-postoperative processes after one-side nephrectomed (N) rats. In these processes alongside with phenolic compounds effect of indomethacin (In) was also studied in order to define an action of phenolic compounds and detect interaction between prostaglandins' function changes and action of phenolic compounds.

The material of investigation was the blood serum of white rats. The phenolic fraction F and indomethacin were injected in animals two hours before and after one-sided nephrectomy.

During the investigation, the changes of mitotic index [7] and cholesterol level were defined by kits of firm "LACHEMA" (Chekh Republic).

RESULTS AND DISCUSSION

On Fig. 1 is shown the results of cholesterol level changes in blood serum under influence of phenolic fraction F and indomethacin on one-side nephrectomed rats on the second and fifth days after operation in comparison with control test. The cholesterol level was increased after nephrectomy (on the 2nd day it was 8,46 and on the 5th – 9,12). At the same time under affect of fraction F, a decrease of these values was revealed (6,58 and 4,86 –on the 2nd and 5th days respectively). It is interesting to note that an indomethacin samely reducing cholesterol level increases it's amount on 5th day, that is probably connected to the fact that indomethacin is a reversible inhibitor of prostaglandin biosynthesis. It is remarkable, that simultaneous injection of fraction F and In after nephrectomy reduces a level of cholesterol on the fifth day but it is less expressed on the second day. We consider that in this case a concurrence for binding site is observed and affect of fraction F is not specific but has much wider spectrum in comparison with indomethacin.

Studying mitotic activity in the conforming tests interesting data have been obtained (Fig. 2). Separately fraction F and indomethacin did not considerably affect mitotic index (for F the mitotic index was 0 and 0,28, for In – 0 and 0,1 on the 2nd and 5th days respectively). The maximal effect of fraction F (2,01 and 2,38) was shown during pre- (F+N) and postoperative (N+F) injections of fraction F. It is remarkable, that simultaneous postoperative injection of fractions F and In - (N+F+In) causes the considerable decrease of mitotic activity (0,9 and 0,33).

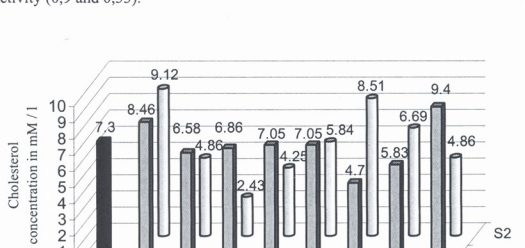

Fig.1. The influence of phenolic fraction of Satureja hortensis and indometacine on the contents of cholesterol in white rats' serum after the second and fifth days of nephrectomy.
C - control, N -nephrectomy, F - phenolic fraction, In – indometacin
S1 - second day; S2 - fifth day
1. C; 2. N; 3. F; 4. F+N; 5. N+F; 6. N+F+In; 7. In; 8. In+N; 9. N+In;

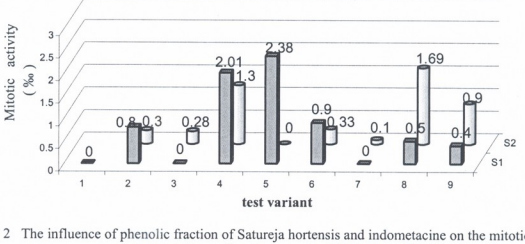

Fig. 2 The influence of phenolic fraction of Satureja hortensis and indometacine on the mitotic activity of white rats' kidney tissue after the second and fifth days of nephrectomy.
C - control, N -nephrectomy, F - phenolic fraction, In – indometacin
S1 - second day; S2 - fifth day
1. C; 2. N; 3. F; 4. F+N; 5. N+F; 6. N+F+In; 7. In; 8. In+N; 9. N+In

So, in our investigation, we have shown that the phenolic compounds from *Satureja hortensis* can be used for prophylaxis and treatment of inflammatory pathology which is connected with dysfunction of cholesterol and also for regeneration - postoperative processes.

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SATUREJA HORTENSIS-დან მიღებული ფენოლური ნაერთების გავლენით ქოლესტერინისა და მიტოზური აქტივობის ცვლილება ცალმხრივ-ნეფრექტომირებულ ვირთაგვებში

მჭედლიშვილი დ., მოდებაძე ი., დავითაია გ.

ბიოფიზიკის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 25.08.2003)

რეზიუმე

ნაჩვენებია, რომ ქონდარიდან *(Satureja hortensis)* მიღებული ფენოლური ფრაქცია F-ის შეყვანა ვირთაგვებში იწვევს ქოლესტერინის დონის მნიშვნელოვან შემცირებას ცალმხრივ ნეფრექტომიამდე და ნეფრექტომიის შემდეგ კონტროლთან შედარებით. ინდომეტაცინის შემთხვევაში კი ეფექტი ვლინდება ოპერაციიდან მხოლოდ მე-2 დღეს, რაც შესაძლოა გამოწვეული იყოს მისი სპეციფიკური და შექცევადი მოქმედებით. ფრაქცია **F** აგრეთვე იწვევს მიტოზური ინდექსის შესამჩნევ ზრდას ნეფრექტომიიდან მე-2 დღეს. ეს ეფექტი უფრო თვალნათელია ფრაქცია F-ის ოპერაციისშემდგომი შეყვანისას. მიღებული შედეგები გვიჩვენებს, რომ ფენოლურ ფრაქცია F-ს ინდომეტაცინთან შედარებით გააჩნია არასპეციფიკური და უფრო ფართო მოქმედების სპექტრი.

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EVALUATION OF OPTIMUM ALGORITHM BY TESTING OF MODIFIED DIRECT DELAYED REACTION

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Abstract

Experiment was performed on white rats using the modified method of direct delayed reaction. The study has been carried out in order to fix complex perception of food in conditions of two feeding racks. Experiments were performed in T-shape labyrinth. Direct delayed reaction method enables to identify maximum delay and study perception process, as well as establish optimum algorithm by means of which the animal makes minimum mistakes and maximally obtains food.

Key words: delayed reaction, memory, algorithm

Introduction

Method of delayed reaction is one of accepted techniques for the study of animals memory. Trace phenomena in the central nervous system has been studied for the first time by Hunter [1]. This method is used for determination of "duration" of a short-term memory. According to Beritashvili [2] delayed reaction is a good tool for specification of both - the short and long term memory.

Objective of our study was to determine quantitative analysis of perception using the method of indirect delayed reaction and identification of maximum delay.

Methods and materials

Experiments were performed on white rats using the method of delayed reaction [3]. Modified method enables us to judge on perception level in the pre-delayed period based on the free behaviour of the animals. The purpose of the study was to fix complex perception of food in conditions of two feeding racks. Experiments were performed in T-shape labyrinth. Food was provided according to the time-spatial program, in conditions of fixed constant delay for each feeding rack and constant interval of food delivery throughout the whole experiment. This program minimizes subjective interference of researcher in the test and enables to study statistics of delayed reaction formation in similar conditions.

Ten tests were performed daily, each test consisting of two phases. The first – delayed reaction during which animal was allowed to move between the feeding racks twice, the second – delayed reaction, during which movement between the racks was restricted. In case of incorrect reaction, when the rack was selected wrongly, the rat returned to the starting compartment without food and the next test started. In delayed reaction it is obligatory to provide food in one of the feeding racks. All reactions must be recorded. Figure 1 means that the rat performs the action within 5 seconds time; "0" means that researches had interfered in the test. In selection of feeding racks "1" means that the animal selects the rack where it has got food previously, "0" – stands for the mistake. As a result, the record represents sequence of "0" and "1", which enables to characterize animals' behaviour and identify algorithm of perception [4,5]. Method of numerical presentation of delayed behaviour representing the algorithm is given in Figure 1.

Results and Discussion

Direct delayed reaction method enables to identify maximum delay and to study perception process, as well as pick out optimum algorithm by means of which the animal makes minimum mistakes and maximally obtains the food (Figure 2). Dynamics of delayed reaction algorithm is given in Table 1.

Analysis of experimental data show that the animals adapt to environment. Frequency for the optimum algorithm occurrence finally increases. If during the first six days the frequency of chaotic algorithm prevails, during the next 6 days the picture changes and frequency of optimum algorithm is predominating (Fig. 3).

Observed variation of behaviour algorithm testifies perception ability in animals. For animals with optimum algorithm maximum delay is 35-40 sec, whereas in case of chaotic algorithm this value totals 15-20 sec.

Suggested method of the delayed reaction study enables probable evaluation of perception dynamics, as well as determination of maximum delay in animals with different level of perception. Analogous method of the study is compared with the results obtained by indirect and direct method possible.

Table 1. Dynamics of delayed reaction algorithm – testing of delayed reaction by means of direct method

test	days									
	1	2	3	4	5	6	7	8	9	10
1	00000	11000	11000	11000	11001	11000	11111	11100	11110	11001
2	01000	10100	11000	10100	11000	11001	11001	11101	11001	10101
3	10010	11110	10100	11111	11001	11101	11001	10101	10111	10101
4	10010	11000	10100	11001	11001	11000	11000	11000	11001	10101
5	11000	11000	11001	10101	11001	11000	11000	11001	11001	11101
6	10100	11110	11110	11001	11001	11000	11000	11001	11111	11101
7	10100	10110	11000	11000	11001	10100	10100	10101	11001	10101
8	11000	11000	11100	11101	11001	10101	10101	11001	11001	10101
9	11000	11000	10100	11001	10101	11001	11001	11001	10101	11001
10	11000	11100	11101	11000	11001	11000	10100	11000	10101	11001

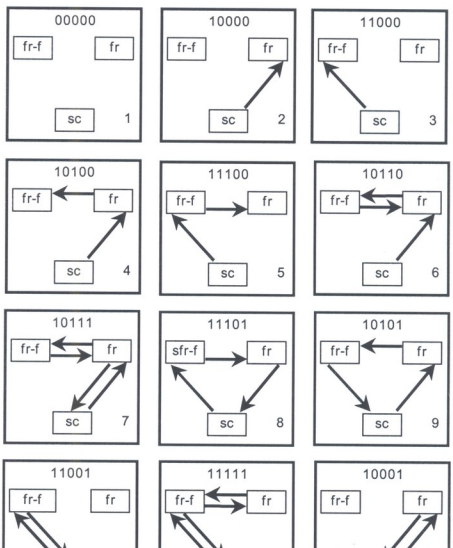
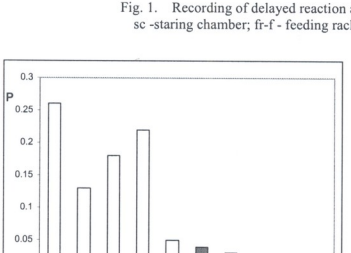
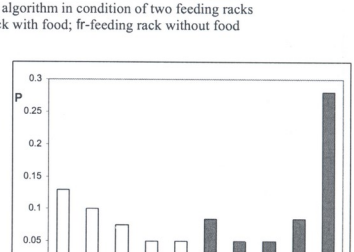


Fig. 1. Recording of delayed reaction algorithm in condition of two feeding racks
sc -staring chamber; fr-f - feeding rack with food; fr-feeding rack without food



days 1 to 6



days 7 to 12

Fig. 2. Frequencies of different algorithms of delayed reaction in obtaining food.
Black columns - stand for optimum, white – for chaotic algorithms

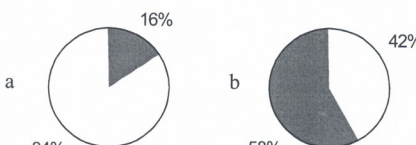


Fig. 3. Chaotic and optimum algorithm realization. a – days 1 to 6; b – days 7 to 12. White colour indicates chaotic, black colour – optimum algorithms.

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ოპტიმალურ ალგორითმთა შეფასება მოდიფიცირებული პირდაპირი დაყოვნებული რეაქციების ტესტირებით

სტამატელი ა., ცაგარელი ს.

ნეირობიოლოგიის სამეცნიერო-კვლევითი ლაბორატორია, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 28.07.2003)

რეზიუმე

ექსპერიმენტის მიზანი იყო თეთრი ვირთაგვების მიერ საკვების კომპლექსური აღქმის დაფიქსირება ორი საკვებურის პირობებში. ექსპერიმენტი ტარდებოდა პირდაპირი დაყოვნებული რეაქციის მოდიფიცირებული მეთოდით. ექსპერიმენტი საშუალებას გვაძლევს დაყოვნების მაქსიმუმის დადგენასთან ერთად დავაკვირდეთ ცხოველთა დასწავლის პროცესს, გამოვყოთ ოპტიმალური ალგორითმი, რომლის მეშვეობითაც ცხოველი მინიმალურ შეცდომას უშვებს და მაქსიმალურად მოიპოვებს საკვებს.

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THE INFLUENCE OF NONSELECTIVE DOPAMINE AGONISTS ON THE RAT BEHAVIORAL MODEL FOR PARKINSON'S DISEASE

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Abstract

It has been shown that a unilateral electrolisically induced dopamine denervation of the striatum constitutes an animal model for Parkinson's disease (PD). The most important features of this model are curved spine, rigidity, tremor, slowness of movement and difficulty in walking. Dopamine supersensitivity of the dopamine receptors evaluate with nonselective dopamine agonists – apomorphine and L-dopa. They induce a strong rotation behavior contralateral to the dopamine-denervated side. This model shows the potential of nonselective dopamine agonists as an active anti-parkinsonian agent. Parkinsonian rats help us to find the best treatment for people with PD.

Key words: Parkinson's disease, apomorphine, L-dopa, rat's behavioral model, turning behavior, blocking study.

Introduction

Parkinsonizm is a collection of neurological features, including tremor, rigidity, akinesia and loss of postural reflexes. Parkinson's disease, the most important cause of this syndrome, is chronic progressive disorder of unknown cause. The pathologic hallmarks of Parkinson's disease are degeneration and loss of the pigmented cells of the *substantia nigra*, with resultant deficiency of the neurotransmiter dopamine in the nigrostriatal pathway [1, 3].

It is accepted that a unilateral 6-hidroxidopamine (6-OHDA) induced dopamine denervation of the striatum constitutes rat's model for Parkinson's disease (PD), or at least a good model to study supersensitive dopamine receptors. This model predicts, that drugs inducing a strong turning behavior contralateral to the dopamine-denervated side are potential dopamine agonists and antiparkinsonian drugs [4].

The aim of the present study is to constitute an animal model for Parkinson's disease electrolisically induced dopamine denervation of the striatum and evaluate the dopamine supersensitivity of the dopamine receptors with nonselective dopamine agonists – apomorphine and L-dopa.

Materials and methods

Male Wistar rats (150-160 g) anaesthetized with 80mg/kg of ketalar i.p., were positioned in a stereotaxic instrument with the skull in the horizontal plane. Coordinates for the lesions were 4,4 mm posterior and 1,2 mm lateral to bregma, and 7,8 mm ventral to the dural surface with the nose bar set –2,3 mm. The electrode of stainless steel of 0,5 mm in diameter insulated with plastic except 2 mm at the tip, was lowered into the brain. The electrode was connected to an electro lesion generator and the indifferent lead was attached

to rat's' finger. 2ma of power applied for 15 sec. After 3 weeks the degree of dopamine denervation was determined by measuring apomorphine-induced rotations (0,5 mg/kg, s.c). Only animals showing at least 50 turns/5 min were included in the experiments, as these animals are known to have achieved 95% of dopamine depletion [2].

2-3 months post-lesion turning behavior was analyzed. The nonselective dopamine D_1D_2 receptors' agonists apomorphine and L-dopa were administered. (Apomoephine 0,05 mg/kg, L-dopa 10 mg/kg s.c.). Only complete and uninterrupted turns were recorded. All drugs were dissolved in 0,9% sterile saline. **n -** number of animals tested was 10 for apomorphine injections. The same group a week later was used for L-dopa injections. One-way ANOVA with Dunnet's post hoc comparison was used for statistical analysis.

Results and discussion

Administration of L-dopa, the precursor of dopamine (DA), and apomorphine produced a pronounced and long –lasting effect (Fig. 1).

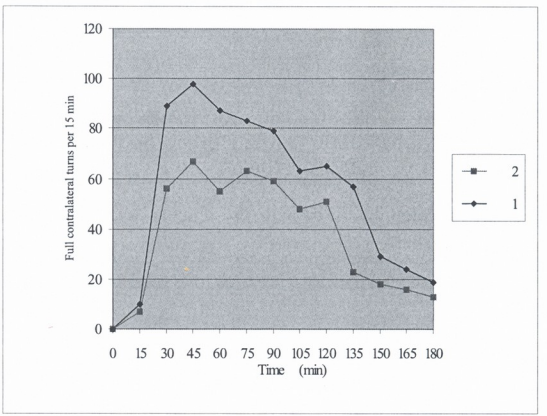

Fig. 1. Pharmacological effect of apomorphine –1 and L-dopa –2 in the model for Parkinson's disease expressed as cumulative full contralateral rotations per 15 min over 3h. n=10 (P<0,01).

Table 1. Bloking study of apomorphine and L-dopa on induced rotation behavior in unilaterally electrolisically lesioned rats. As antagonist were used: the DA D_2 antagonist haloperidol (Hal), and the DA D_1 antagonist SCH 23390 (SCH).*

Treatment (Mg/kg s.c.)	Vehicle	Hal	SCH	Hal+SCH
L-dopa (10)	439 (±12)	312(±55)	210 ((±30)	35(±10)
Apomorphine (0,05)	501(±48)	435(±30)	306(±28)	135(± 20)

* Rats were administered 0,3 mg/kg of haloperidolip and 0,3 mg/kg of SCH23390 one minute before administration of the DA agonist. The results are means ± SEM (standard error means) of 4 animals per group. The same group of rats was used per treatment.

This clearly shows, that these drugs are active enantiomers, and produce effects that were mediated by both DA D_1 and DA D_2 receptors. The involvement of both DA D_1 and DA D_2 receptors was further confirmed by the results of the selective blocking studies in the Parkinsonian rat's model (Table 1). It is necessary to antagonize both DA D_1 and DA D_2 receptors to almost completely block the behavioral effects of L-dopa and apomorphine.

In conclusion this study shows that electrolisically induced dopamine denervation of the striatum constitutes the rat model for Parkinson's disease to study supersensitive dopamine receptors. This model shows the potential of nonselective dopamine agonists as an active anti-parkinsonian agent. Parkinsonian rats help us to find the best treatment for people with PD.

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არასელექტური დოფამინური აგონისტების გავლენა პარკინსონის დაავადების მძრნე ვირთაგვების ქცევით მოდელებზე

ორმოცაძე ნ., სიხარულიძე ნ., მიქავა რ., მელია ნ., დავითაშვილი დ.

ადამიანისა და ცხოველთა ფიზიოლოგიის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 28.07.2003)

რეზიუმე

ნიგროსტრიალური ტრაქტის ცალმხრივი ელექტროლიზური დაზიანების პირობებში ვისტარის ხაზის თეთრ ლაბორატორიულ ვირთაგვებში მიღებული იქნა პარკინსონის დაავადების ანალოგიური ქცევითი მოდელი. ვირთაგვებს აღენიშნებოდათ ხერხემლის გამრუდება, თავის ასიმეტრიულობა, რიგიდულობა, ჰიპოკინეზია, ტრემორი. ასეთი მოდელები წარმოადგენენ საუკეთესო ობიექტებს დოფამინური სუპერსენსიტიურობის შესასწავლად და ანტიპარკინსონული ნივთიერებების გამოსავლენად. შესწავლილი იქნა არასელექტური დოფამინური აგონისტების – აპომორფინისა და L-დოფას გავლენა, რომლებიც აღძრავენ ცხოველის ძლიერ ბრუნვით რეაქციას დაზიანების საწინააღმდეგო მხარეს. გამოთქმულია მოსაზრება, რომ აღნიშნული ნივთიერებები ფარმაკოლოგიურ ეფექტს ახდენენ ორივე დოფამინური D_1 და D_2 ქვეტიპის რეცეპტორების გააქტივების შედეგად. აღნიშნული თვალსაზრისი განმტკიცდა ბრუნვითი ქცევის შეკავების ცდებში, დოფამინური ანტაგონისტების გამოყენების შედეგად.

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Zoology

ROSE SOFT SCALE (*HEMIPTERA: COCCIDAE*) AND ITS PARASITOID IN ISPARTA PROVINCE (TURKEY)

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Abstract

Microterys bellae Trjapitzin (Hymenoptera: Encyrtidae), a parasitoid of Rose Soft Scale, *Rhodococcus perornatus* (Cockerell & Parrott) (Hemiptera: Coccidae) has been found in Turkey for the first time. As the some characters of *Microterys bellae* was given unclear a detailed description and illustrations are provided. Observations on its dynamic relationships and effectiveness as a natural enemy of Rose Soft Scale also are made for the first time.

Key words: *Rhodococcus perornatus, Microterys bellae,* Turkey

Introduction

Rose oil is one of the most important agricultural exports from Turkey. Several pests and diseases cause economic losses in oil roses (*Rosa damascena* L.) and *Rhodococcus perornatus* (Cockerell & Parrott) (Hemiptera: Coccidae) (Rose Soft Scale) is a key pest of this crop in Turkey. The *Rhodococcus perornatus* infest the branches and trunks of its host plants, particularly oil roses, which can be seriously damaged and may even die. More often, however, the plants become defoliated due to the accumulation of sooty moulds growing on the honeydew [1]. It has been recorded in the Palaearctic region from Austria, Bulgaria, Hungary, Italy, Moldova and some parts of Russia [2, 3]. It was first reported from Turkey in 1999 [4] and its distribution and economic impact have been increasing steadily; however, no detailed study of this pest in Turkey has been made.

There are some reports on the natural enemies of Rose Soft Scale [1, 6, 7, 8], but no detailed study has been made of their effectiveness or on their use to regulate Rose Soft Scale populations. The aims of this study were to identify the parasitoids of *R. perornatus* on oil rose in Turkey, and to investigate the population development of the scale and its parasitoids, and the efficacy of these parasitoids in Isparta province. The effectiveness of *Microterys bellae* Trjapitzin (Hymenoptera: Encyrtidae) as a regulator of Rose Soft Scale populations had not been investigated prior.

Materials and methods

Oil rose areas in Turkey were regularly surveyed during the 2001 growing season to document the distribution of rose soft scale. Population fluctuations of the scale insect and its parasitoid were investigated by taking samples from 3 fields every 15 days. From each field, 50 oil rose twigs were taken for laboratory examination. The material was collected in each site from 10 bushes, which were chosen randomly. The number of Rose Soft Scales on 10 cm of each branch were counted. Each sample was examined under a

stereomicroscope and the rate of parasitism was determined as a percentage of the sample with emergence holes on them. Some samples were kept in boxes in order to obtain the emerging parasitoids for identification.

The phenology of the scale was studied by recording the average proportion of each sample of 200 scale insects at each developmental stage. Scale population density was recorded as the mean number of scales per 10 cm of branch. The percentage of scale insect damage was established using the formula P=B.100/a, where P - percentage of damage, B - proportion of damaged plants in the samples, and a - total number of scale specimens [9]. This formula was also used to determine the role of parasitoids in the regulation of numbers of sap-sucking insects. The rearing of the parasitoids from coccids was conducted by generally accepted methods [10,11].

Results and Discussion

Rose Soft Scale was found in almost every field examined in the survey area. There was one generation per year, with overwintering as second stage nymphs on branches or in other covert places; development resumed in March. Fecundity was high, with each female producing 500 - 800 eggs. Larvae hatched from the eggs in June-July and crawled over the plants to find feeding sites on the branches. Saprophytic fungus (sooty mould) developed on sugary excreta (honeydew) fouling the plant surfaces, blackening the green parts of the plants and significantly decreasing plant quality and may even kill.

The phenology studied since 2001-2002 was slightly different from the phenology documented by other authors [1]. The development and population dynamics of Rose Soft Scale in Isparta province in 2002 is given in Fig. 1.

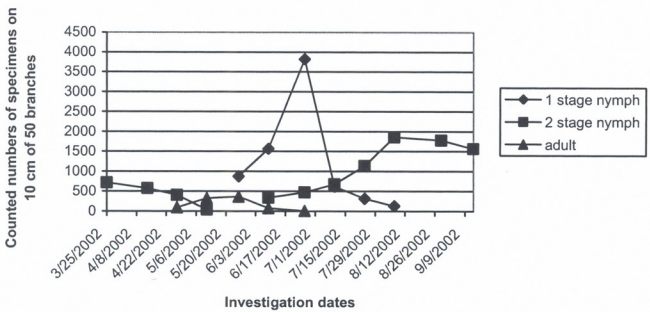

Fig.1 The development of Rose Soft Scales (*Rhodococcus perornatus*) since 2002.

Heavy infestations were observed prior to the end of 2001. Infestation and parasitisation rates for 2001 in the field are given in Table 1. In 2002 parasitoid was not registered.

Table 1. Infestation rates of *Rhodococcus perornatus* on oil rose, and *Microterys bellae* in Rose Soft Scale, in the field in southwest Turkey.

	Gölcük	Kuleonu	Isparta
Percentage of rose plants damaged	98%	98%	96%
Average no. of ovipositing female scales per 10cm of stem	48 (range 1-110)	35 (range 1-88)	26 (range 1-41)
Percentage of female scales parasitized	0.9% (9 out of 985)	1.96% (12 out of 612)	0% (0 out of ?)

Our observations showed that the cause of the pest outbreak was the arrival of *R. perornatus* in Isparta province without its natural enemies. The local enemies have not adapted to the feeding on Rose Soft Scale yet. During the study only one parasitoid species was found on Rose Soft Scale; this was identified as *Microterys bellae,* a new addition to Turkey's fauna. Previously, this parasitoid was known only from a small part of the North Caucasus (Kabardino-Balkaria) from the same species of scale insect host [7, 12].

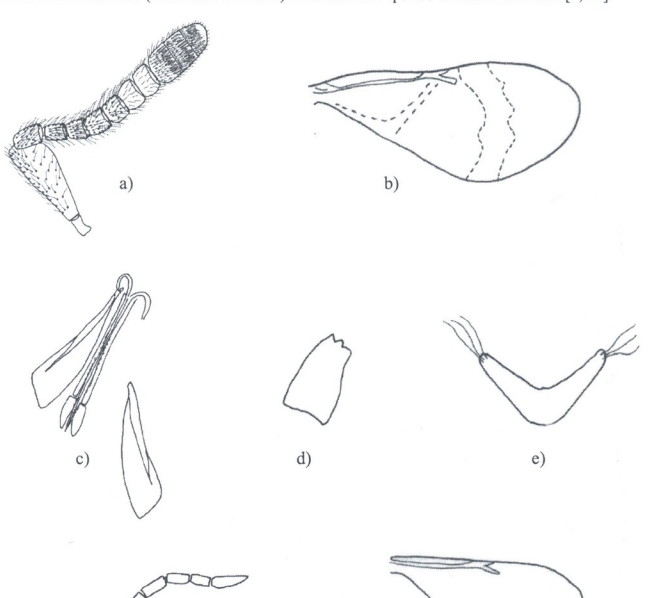

Fig. 2. *Microterys bellae*, ♀; a) right antenna; b) right forewing;c) ovipositor; d) mandible; e) ninth tergite; ♂; f) left antenna; g) right forewing

Description of *Microterys bellae* Trjapitzin. Female: Frons and vertex twice as long as wide. Ocellar triangle 60°. Cheeks approximately as long as eyes [¾ units]. Distance between antennal toruli twice as long as distance from antennal toruli to mouth margin. An antenna is illustrated in Fig. 2a. Scutellum with a slight projection, more or less as wide as long (13:14) or a little shorter. Mesoscutum almost as long as scutellum, slightly short, wider than long (5:3). Forewing width is almost twice shorter than width (Fig. 2b). Mid-tibial spur slightly shorter than first tarsal segment (6:5.5). Inner plate of ovipositor 43.5 times longer than its width at the narrowest point, and 25 times longer than its width at the widest place. Length of outer plates of ovipositor about 3.6-3.7 times longer than wide (Fig. 2c). Mandible three dentate (Fig. 2d). Angle of ninth tergite 90° (Fig. 2e). Frons and vertex dark yellow. Entire body dark and yellowish. Antenna also dark yellow. Fourth segment of funicle slightly pale, then segments 1-3 and 5-6 white. Antennal club dark brown.

Mesoscutum, scutellum and axilla with dark green-silver lustre. Tegulae and sides of axilla slightly yellowish. Metanotum and propodeum dark black, with blue-green lustre. Pronotum dark blackish or brownish, without lustre. Forewings dusky, with one slightly curved, light line in the front one-third part. This line 3.75 times shorter than length of fore wings. Dorsum of abdomen with black-violet-silverish lustre, venter with goldish lustre and white bristles; legs dark yellow; middle coxa dark brown; hind coxa darker than middle coax; middle coxa, hind femur and hind tibia are equally dark. Mesoscutum, scutellum and axilla with same structure; Mesopleura dark yellow; Mesoscutum and scutellum covered by short, black bristles. Body length: 1.8-2.4 mm.

Male: Frons and vertex as wide as long. Ocelli create an equilateral triangle. Antenna and forewings are illustrated (Fig. 2f-g). Head and thorax green with metallic lustre. Scape yellow; pedicel dark; segments of funicle dark yellow. Mesopleura, middle and hind coxae dark yellow. Fore legs yellow, hind legs with dark tibia and femora. Abdomen with goldish-violet lustre. Body length: 1.75-1.9 mm.

Material examined: 5-20.V. 2001, Ex *Rhodococcus perornatus* on *Rosa damascena* 33♀, 22♂, Gölcük; 12.V.-5.VI. 2001, Exed from *Rhodococcus perornatus* on *Rosa damascena* 2♀, 1♂, Kuleonu.

Growers commonly use broad-spectrum insecticides (e.g. methidathion) against Rose Soft Scale, which are harmful to its natural enemies. However, insecticides are effective only against the immature scales, which are covered by only a thin scale-like cover; each adult female is completely enclosed in a thick, sclerotized "puparium" that is impenetrable to insecticides. In Isparta province, the effectiveness of parasitoids in the suppression of pests is decreased by the used agricultural methods , which minimize the parasitoid population. In the longer term it often causes recurrent pest outbreaks due to the buildup of pesticide resistance and decimation of beneficial natural enemy population. We think that it would be useful to create several refuges to promote the natural development of host-parasitoid relations and effectiveness of parasitoid. The ideal solution to the pest problem is to conserve natural host-parasitoid relations and avoid any disruption of these processes. Pesticide use is also expensive, hazardous to humans and wildlife, and is a major cause of environmental pollution. Alternatives to chemical control, such as biological control are urgently needed [5] and can play an important role. Our studies on the solution of this problem will continue to provide more complete data resources. We hope that *M. bellae* can be an effective natural enemy of *R. perornatus*, and that it should be used in the biocontrol of Rose Soft Scale in Turkey.

Acknowledgements

We would like to thank to Dr S. Ulgenturk for helping to identify the scale insect, and Dr G.W. Watson for her suggestions. We are also grateful to The Scientific and Technical Research Council of Turkey (TUBITAK) and NATO PC-B program Ref: B.02.1. BAK.009.00.00/386/985 and Ref: B.02.1.BAK.009.00.00/562/1439 for supporting this work.

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ვარდის რბილი ცრუშარიანა (*Hemiptera: Coccidae*) და მისი პარაზიტოიდი ისპარტას პროვინციაში (თურქეთი)

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(მიღებულია 23.07.2003)

რეზიუმე

თურქეთისათვის პირველადაა აღრიცხული ვარდის რბილი ცრუშარიანას *Rhodococcus perornatus* (Cockerell & Parrott) (Hemiptera: Coccidae), პარაზიტოიდი *Microterys bellae* Trjapitzin (Hymenoptera: Encyrtidae). რადგან *Microterys bellae*–ს ზოგიერთი ნიშანი არ არის დაზუსტებული, ამიტომ მოცემულია დეტალური ხელახალი აღწერა და ილუსტრაციები. დაკვირვები პირველადაა წარმოებული მასპინძლისა - პარაზიტოიდის ურთიერთკავშირსა და დინამიკაზე.

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Ecology

LICHENS - THE INDICATORS OF THE AIR POLLUTION OF TBILISI

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Abstract

The epiphytic lichen flora of Tbilisi has been studied in order to reveal lichen species, which can be successfully used for air quality monitoring. Appropriate measures (species richness, frequency of occurrence of individual species, total cover) have been recorded at each study location within the city. The investigation has shown that about 25 epiphytic lichen species can be used for air quality monitoring in Tbilisi. The species associated with zones of high, moderate and low pollution have also been distinguished. The investigation has also shown that *Physciopsis adglutinata* is associated with the zone of high pollution and *Candelaria concolor* and/or *Xanthoria substellaris* together with *Ph. adglutinata* are characteristic to the zone of moderate pollution. In areas of the low pollution the level of the number of epiphitic species increases to 20-30 and more; frequency of occurrence of individual species and total cover also remarkably increase (the latter often comprising 80-90% on certain exposures).

Key words: epiphitic lichens, bioindicators, air quality monitoring.

Introduction

Lichens have been successfully used for air quality monitoring in many countries of the world. They are particularly useful in indicating pollution loads over long periods [2, 3, 4, 6, 7]. Studies can be in relation to point emission sources such as power plants and smelters, a general source area such as an urban area or industrial complex, or as a means of producing baseline data of a previously unsurveyed site or in pre-development appraisals [2].

As the correlation between the air pollution level and the state of the lichen flora and vegetation of Tbilisi has not been investigated so far, the aim of the present research is to compile baseline data for future monitoring studies.

We have studied the lichen flora of differently polluted parts of Tbilisi, where vehicle exhaust is the main source of air pollution nowadays. The present investigation has revealed about 25 species of epiphytic lichens useful for air quality monitoring studies in Tbilisi [1]; species associated with zones of high, moderate and low pollution have also been distinguished.

Materials and methods

Lichens were investigated at a number of locations differed in the extent of air pollution (Dighomi, Didube, Saburtalo, Vake, Mtatsminda, Orthacala, the city center).

Samples were taken from 44 species of woody plants.

The species lists were compiled for each study location. Appropriate measures: species' richness, frequency of occurrence of individual species and total cover were recorded and the health of lichen thalli was assessed. Finally, the data obtained from differently polluted locations were compared.

Results and discussion

Three zones of different pollution level have been distinguished in Tbilisi: the linear zone of the high pollution level includes the areas, which are the nearest to the central roads and streets (Orthachala, the Liberty Square, Rustaveli, Chavchavadze, Tseretheli Avenues, Vake-Saburthalo Road); the zone of the moderate pollution includes the parks located near the areas of high pollution level and the zone of the low pollution level covers the peripheral parts of the city distant from the traffic.

The zone of the high pollution. Epiphytic lichens have almost completely disappeared at locations across the roads and streets, which are strongly polluted with the vehicle exhaust; however, individual, often damaged specimens of *Physciopsis adglutinata* are scattered over this strongly polluted area.

The zone of the moderate pollution. In parks located near the pollution sources two species: *Candelaria concolor* and/or *Xanthoria substellaris* are often present besides *Physciopsis adglutinata.* The frequency of occurrence of *Physciopsis adglutinata* can be evaluated as 80% there and that of *Candelaria concolor* and *Xanthoria substellaris* - as 40%. The total cover amounts 70-90% on certain sides of tree trunks. However, in the Didube district, where SO_2 concentration amounts 190 mkg/m^3 [5] only *Physciopsis adglutinata* is present even in green plantations and although its cover is about 70% on some tree trunks, the frequency of occurrence is too low.

Table. Epiphytic lichen species indicating different pollution level in Tbilisi

AREAS OF HIGH POLLUTION	*Physciopsis adglutinata*
AREAS OF MODERATE POLLUTION	*Physciopsis adglutinata* *Candelaria concolor* *Xanthoria substellaris*
AREAS OF LOW POLLUTION	*Xanthoria parietina* *Xanthoria ulophylodes* *Physcia adscendens* *Physcia aipolia* *Physcia stellaris* *Physconia pulverulenta* *Parmelia acetabulum* *Lecidea glomerulosa* *Lecanora atra* *Lecanora carpinea* *Lecanora hageni* *Lecanora rugosella* *Lecanora subrugosa* *Candalariella aurella* *Caloplaca pyracea* *Caloplaca cerina* *Rinodina pyrina* *Lepraria aeruginosa*

Thus, *Physciopsis adglutinata* should be considered as the most tolerant species within the study area and is, therefore, associated with the strongly polluted zone, while the presence of *Candelaria concolor* and / or *Xanthoria substellaris* (on the background of too low frequency of occurrence of other species) indicate moderate pollution.

In the moderately polluted zone the following species also appear: *Xanthoria parietina, Physcia adscendens, Physconia pulverulenta, Lecidea glomerulosa, Lecanora hageni, Candelariella aurella, Caloplaca pyracea, Rinodina pyrina*, but the frequency of occurrence of each of these species do not exceed 25-30%.

The zone of the low pollution. In the study sites (located in the Tbilisi botanical Garden, the Mthatsminda Park, the Vera cemetery, the surroundings of Turtle Lake and Lisi Lake, the Vere gorge), which

are comparatively distant or isolated at some extent from pollution sources the quantity of epiphytic species rises to 25-30; the coverage and frequency of occurrence of individual species also considerably rises at these locations.

The following species are present in the zone of low pollution with the frequency of occurrence more than 40%: Physcia aipolia, Ph. stellaris, Ph. adscendens, Ph. hispida, Physconia pulverulenta, Xanthoria parietina, X. ulophylodes, Parmelia acetabulum, Lecidea glomerulosa, Lecanora atra, L. carpinea, L. hageni, Candalariella aurella, Caloplaca cerina, C. pyracea, Rinodina pyrina, etc.

Thus, the present investigation has revealed the epiphytic lichen species, which may be recommended as bioindicators in air quality monitoring studies in Tbilisi.

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ლიქენები – თბილისის ატმოსფეროს დაბინძურების ინდიკატორები

ბაცაცაშვილი ქ., ჭელიძე ნ.

ბოტანიკის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 28.07.2003)

რეზიუმე

ჩატარებულია სამუშაო ეპიფიტური ლიქენების იმ სახეობათა გამოვლენის მიზნით, რომელთა გამოყენება შესაძლებელია ქ. თბილისის ატმოსფეროს დაბინძურების დონის ბიოინდიკატორებად. შესწავლილია ეპიფიტური ლიქენების სახეობრივი მრავალფეროვნება, სახეობათა შეხვედრილობის სიხშირე, დაფარულობა, თალუსების მორფოლოგიური მდგომარეობა. დადგენილია, რომ ქ. თბილისის ატმოსფეროს დაბინძურების დონის ინდიკატორებად შესაძლებელია ეპიფიტურ ლიქენთა 25-მდე სახეობის გამოყენება. გამოყოფილია ძლიერ, ზომიერად და სუსტად დაბინძურებული ზონებისათვის დამახასიათებელი სახეობები. ძლიერ დაბინძურებულ ზონაში აღნიშნულია მხოლოდ *Physciopsis adglutinata*; ზომიერად დაბინძურებულ ზონაში – *Candelaria concolor* და/ან *Xanthoria substellaris Ph. adglutinata*-სთან ერთად; ხოლო სუსტად დაბინძურებულ ზონაში აღნიშნულია როგორც სახეობათა რაოდენობის ზრდა (20-30-მდე და მეტად), ისე ცალკეულ სახეობათა შეხვედრილობის სიხშირისა და დაფარულობის ხარისხის (გარკვეულ ქსპოზიციებზე – 80-90%-მდე) მნიშვნელოვანი მატება.

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FUNGI OF *GENUS METARHIZIUM* AS PATHOGENS ATTACKING LOCUST

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ABSTRACT

The need for alternatives to chemical pesticides makes fungal biological control a priority. As a result of researches were revealed and studied pathogenic fungi attacking locusts. It was identified that epizootic of Italian and Moroccan locusts in Kakheti was caused mostly by *Entomophthora and Beauveria*. Besides the first fungi of *Genus Metarhizium* were revealed in Georgia. Their morphological features were studied. Identification of species was carried out on the base of conidial and phialide morphology. The pathogenicity towards Locust is studying.

Key words: *Metarhizium ansopliae, Metarhizium sp.*, fungal pathogens, entomopathogenic fungi, phialide, pesticides.

Introduction

Pollution of environment by chemical pesticides needs to be significantly reduced. In recent years a considerable interest has received usage of fungal pathogens for the control of crop pests, which is environmentally friendly alternatives to chemical pesticides. A number of isolates of *Beauveria, Entomophthora, Ashersonia, Verticillium, Cephalosporium* has been shown to be pathogenic for pests.

Many authors indicate on Metarhizium as highly pathogenic fungi for locust and grasshoppers and are being developed as mycoinsecticides [1]. Recent outbreak of Italian and Moroccan locust caused a great damage to crops and pastures in Georgia [2]. In 1998 and 2000 years epizootic was noticed. About 65-70% of the population of Calliptamus italicus was infested [3]. Microscopic analysis of infested locusts, fungal isolation and culturing established, mostly fungi *Entomophthora grylli* and *Beauveria bassiana* caused the epizootic. The fungi of genus Metarhizium were identified as well. It is the first case of identification of genus Metarhizium in Georgia.

A. Evlakhova mentions two species of this genus: *M.anisopliae* and *M. bruneu*, which probably are variety of *M.anisopliae*. It was founded on plant hoppers in the Philippines [4].

A total of 3 species have been described in the *genus Metarhizium: M. anisopliae* (Metschn.) Sorokin, *M. flavoviridae* (described by Gams and Rozsypale 1973) and *M. album* first described by Petch [5].

A number of authors emphasize that *M. anisopliae* is entomopathogenic for many pests (beetles, acridoids, cicadas, white flies, etc.). Promising results using *M. anisopliae* against acridids in Niger, Australia, Mexico, Madagascar indicate the potential for use of mycopesticides based on Metarhizium [6,7]. Mycopesticides on the base of *M.anisopliae* are used against pests in many countries under various

commercial names: as Bio-1020 in Germany, Biogreen in Australia, Cobican in Venezuela, Metaboil in Brasilia, Bio-Path and Bio-Blast in the USA, etc. The producer is indicated active substance on the label. Mainly spores are mentioned as an active substance, except German product, where mycelium is indicated as an initial substance.

MATERIALS AND METHODS

Fungal isolates and culturing. Infected and dead locusts were collected in natural conditions. Microscopic analyses, fungi isolation and culturing were carried out. Media used in culturing was malt agar. Isolates were grown at 25^0 C for up to two weeks.

Infection of Calliptamus italicus. Hoppers and adults of *Calliptamus italicus* (*Orthoptera*) were inoculated at 28^0 C. After death cadavers were placed in moist filter paper in a Petri dish to promote sporulation. Morphological features of isolated fungi were studied by means of microscopic analysis.

RESULTS AND DISCUSSION

Two groups of isolates of *Metarhizium* were isolated from dead locusts. The first group was formed two types of isolates: dark green conidia on rapidly growing colonies and light green conidia on slow growing colonies. Isolates of the second group were flesh- brown colored.

Their morphological characteristics are given below.

1. *Metarhizium anisopliae* causes disease and mummification of locust. Their body often is covered of white – green coating, which represents fungus fertility. Microscopic analysis of infected specimen established the hyphal mycelium in the abdominal and prothorax tissues of insects. At the beginning layer of mycelium on malt agar is colorless or white. Later it acquires felt consistence and greeny color, airy mycelium is not formed. Straight – standing, rarely curved, stick – shaped conidiophores were formed on the surface of colony. Their length is 26.6 – 35.1x3.8-4.2 μm. Phialides often-formed on dichotomously differentiated conidiophores. The length of phialide is 11.4-10.0 μm.

Greeny color and character of surface colony is conditioned by excessive sporulation. The spores are unicellular, oval or a bit oblong. At the beginning they are colorless, later become grey – greeny color, the size of oval spores is 3.8-4.5 μm., the length of oblong spores is 5.7-6.5x3.8-4.6 μm. (Fig. 1).

Metarhizium was isolated from *Calliptamus italicus* in 2000 (Kakheti region, Georgia).

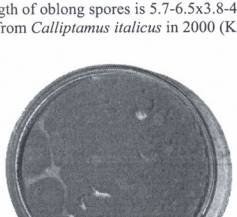

Fig. 1. Colony of *Metarhizium anisopliae* isolated from *Calliptamus italicus.*

2. Metarhizium sp. damages locusts. Hyphal mycelium is disseminated in the insect tissues. At the first the layer of mycelium is colorless, after 4-5 days it acquires light-brown color; the surface becomes felty and friable that is caused by abundant sporulation. Hyphae are colorless, multicellular with rich substance, the diameter is 3.8-10 μm. Spores are in a great number, oval, egg-shaped, colorless, but in mass are dark yellow colored. The sizes are 5.7-11.4x5.7-8.5 μm.

Spores are forming on stick-shaped conidiophores, which branch at the apex on 2 or 3 phialides. Bead - shaped spores easily dissociate. Phialides have cylindrical shape or are a bit wider at base. The length is 15.2-22.8 μm.

It was isolated from locust in 2002 (Tbilisi suburb, Georgia).
In the table 1 is given comparative morphology of *Metarhizium* isolates

Table 1. Comparative morphology of *Metarhizium* isolates

N	Name of Species	Original host	Sizes in μm			Color of colony
			conidiophores	phialides	Spores	
1	*Metarhizium anisopliae*	*Calliptamus italicus (Orthoptera)*	26.6-35.1	11.4-19.0	5.7-6.5 x 3.8-4.6	first colorless, after 4-5 days acquires greeny color
2	*Metarhizium sp.*	*Calliptamus italicus (Orthoptera)*	19.0-28.1	19.0-21.3	5.7-11.4 x 5.7-8.5	at the beginning colorless, later acquires flesh- brown color

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გვარ *metarhizium* –ის წარმომადგენელი, როგორც კალიების დაავადების გამომწვევი

წაქაძე თ.[1], აბაშიძე ე.[2], სამადაშვილი დ.[2], ოდიკაძე ქ.[2]

[1] *ლ. ყანჩაველის სახ. საქართველოს მცენარეთა დაცვის სამეცნიერო-კვლევითი ინსტიტუტი*
[2] *ეკოლოგიის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი*

(მიღებულია 29.08.2003)

რეზიუმე

გამოვლენილი და შესწავლილია კალიების დაავადების გამომწვევი სოკოები. დადგინდა, რომ საქართველოში იტალიური და მაროკოს კალიების ეპიზოოტია გამოწვეული იყო გვარ *thomophthora* და *beauveria*-ს წარმომადგენლებით. პირველად საქართველოში გამოვლინდა ენტომოპათოგენური სოკოები *metarhizium*-ის გვარიდან. შესწავლილია მათი მორფოლოგიური თვისებები. მიმდინარეობს კვლევა კალიების მიმართ მათი პათოგენობის დადგენის მიზნით.

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Plant Physiology

DETERMINATION OF BIOLOGICALLY ACTIVE PURE AUXINS, GROWTH INHIBITORS AND LECTINS IN THE GROWING ORGANS OF MULBERRY TREE

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Abstract

An activity of exogenic Auxins and growth inhibitors in leaves and inflorescent of mulberry at the different stages of ontogenesis (Apical bud, preflorescence, florescence and fruitage) have been studied. Maximum activity of stimulating substances is revealed in leaves and inflorescent during the growing period (300-225%) and in the following phases their content reduces (180-150%). The presence of correlation between the lectin content and the activity of endogenic Auxins in growing leaves and inflorescent is shown.

Key words: mulberry, Auxin, lectin, distribution, growth.

Introduction

One of the main branches of plant physiology is the study of phytohormone influence on the regulation of plant life cycle. Interest in this process is caused by the fact that hormone–inhibitor balance determines physiological and morphogenetic processes in the plant.

According to the recent literature data, besides of the 5 main phytohormones known so far (Auxins, gibberelines, cytochinins, Ethylen, Abscyze acid, Jasmine acid, brasinolydes) new class of hormones was discovered, that includes small signaling molecules of polypeptide nature [1]. At present, we can say that the physiologically active polypeptide signaling is an emerging field in plant biology, particularly in plant self-defense, fertilization, growth and development. According to basic data, analogy between nature and molecular mechanisms in the action of plant and animal hormones could be found. Also must be mentioned, that all hormones found in animal cells and their receptors are glycoproteins, main parts of which are lectins. Thus, consideration of plant lectins as plant hormones is of great interest and represents a new approach in plant physiology.

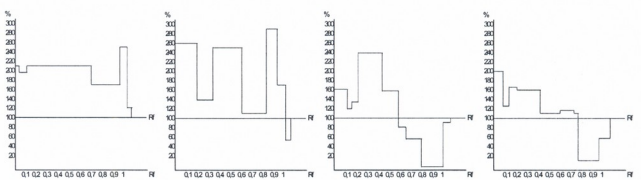
Material and methods

Following from above, it was very important to study the dependence between the activity of endogenic Auxins and growth inhibitors and content of the lectins in growing leaves and florescent of mulberry tree at the different stages of ontogenesis (apical bud, preflorescence, florescence and fruitage).

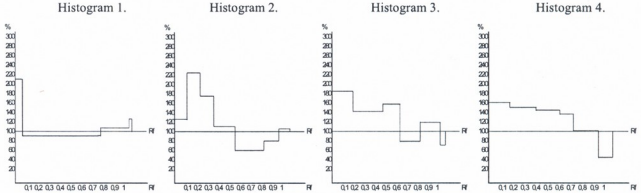
An activity of Auxins and growth inhibitors has been studied by using methods suggested by V. Keffel and others [2]. An activity of hormones was checked by biotest on wheat coleoptiles scraps growth [3]. Endogenic Auxins and growth inhibitors in the mixture were separated by solvent of butyl/vine acid/water (40:12:28). Extraction of proteins with lectin activity was performed by decreasing activity of K^1-phosphate buffer pH7.4. Lectin activity was determined by hemagglutination test on trypsin-treated rabbit erythrocyte [4]. The protein concentration was estimated by Lowry method [5].

Results and discussions

It has been shown, that in the leaves of juvenile growing mulberry (2 years old) and also in the apical leaves of mulberry tree, vegetative growth activity was determined only by stimulatory compounds (190-240%). The substance with Rf (distance from start to mark / distance from start to front in the chromatogramm) 0,9-0,96 showed maximum activity 240% (Histogram 1). With changes of the content of the growth inhibitors in the leaf bud during its development the picture is somehow varies. At this stage one inhibitor (growth was inhibited by 55% - Rf 0,95-1.0) and 3 substances with high stimulatory nature (growth activity reached 260-250 and 295%) were identified, with Rf 0-0,16; 0.28-0,54 and 0,78-0,88; respectively (Histogram 2). Thus at this especially active stage of leaf growth high activity of growth substances was revealed. Mature leaves contain more growth inhibitors (growth hinged by 95-90%-Rf 0,76-0,95; 0,72-0,9 receptively (Histogram 3, 4)), in comparison with growing leaves, which is in good accordance with the picture of growth inhibition process. Thus there is an obvious correlation between the growing process and the content of growth substances in apical leaves of mulberry tree.



Histogram 1. Histogram 2. Histogram 3. Histogram 4.



Histogram 5. Histogram 6. Histogram 7. Histogram 8.

It is known that the infibition in vegetative growth activates the process of the formation and growth of reproductive organs. Though, in some plants these processes pass together. The peculiarity of hormonal regulation of some plants is that the same hormone complex serve growth, as well as florescence process. Only the relatively ratio between the growth stimulators and inhibitors changes. Giberellin has the most

important role in florescence but the same process is dependant on the ratio of Auxins and inhibitors. Therefore the ratio of the Auxins and growth inhibitors in the apical of mulberry tree was also studied.

The similar picture was shown in the apical inflorescence of mulberry by the activity of growth substances. Maximum activity of growth substances was 215-225% (Rf 0-0.33 and 0.05-0.19 (Histogram 5,6,7)) and maximum activity of inhibitors was about 55% (Rf-0.88-1.0 (Histogram 8)).

An activity of growth substances in the apical leaves and inflorescent at different stages is shown in Fig.1. As we can see the picture reveals a certain order in these processes: an activity of stimulating substances in the leaves and inflorescent is at maximum (295-225%) during the growth period (first week period of the appearance of inflorescent and leaves from the apical bud) when at the following phases their content reduces (180-150%). An activity of the substances is at especially high rate in apical leaves. With the accomplishment of growth process of inflorescent as well as leaves the content of the inhibitor substances is revealed with the maximum activity in apical leaves (95%).

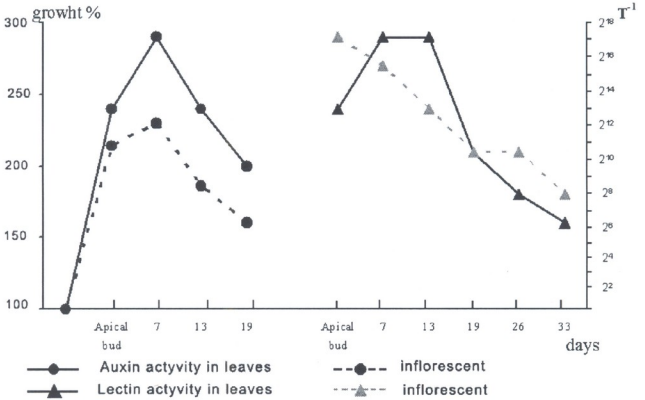


Fig. 1 The change of endogenic Auxins and lectin content in the growth period in the apex leaves and inflorescent of mulberry.

Interesting data are obtained concerning lectin content. These results are shown in Fig. 1. As we can see, lectin content (lectin content in hemagglutination units: sum. protein / hem. activity) in apical leaves and inflorescent was much higher (524288) during the active growth period. Whereas to the end of growth processes in the leaves and fruit formation from inflorescent the lectin content (64) sharply reduces (about 8000 times).

Thus, the presence of positive correlation between the lectin content and the activity of growth stimulating hormones is shown. Accepting the lectin as a primary stimulator or the hormone is very difficult, but as it is shown at Fig.1- decreasing hormonal activity precedes the reduction of lectin activity. Therefore it is assumed that the high content of lectins and their accumulation in the growing organs of mulberry tree could be connected to the activity of growth stimulatory hormones, particularly Auxins. The further study of the molecular mechanisms of the activity of mulberry lectins in the above-described processes is the purpose of our future investigation.

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თუთის მზარდ ორგანოებში ბიოლოგიურად აქტიური თავისუფალი აუქსინების, ზრდის ინჰიბიტორების და ლექტინების შემცველობის შესწავლა

ხურციძე ე., ბაგრატიონი ნ., გერკეული მ., ოსეფაიშვილი ი., ალექსიძე გ.

მცენარეთა ანატომიისა და ფიზიოლოგიის კათედრა, თბილისის ივ. ჯავახიშვილის სახელობის სახ. უნივერსიტეტი

(მიღებულია 28.07.2003)

რეზიუმე

შესწავლილია ენდოგენური აუქსინების და ზრდის ინჰიბიტორების აქტივობა თუთის ფოთლებსა და ყვავილედებში, ონტოგენეზის სხვადასხვა (კენწრული კვირტის, ყვავილობის წინა, ყვავილობის და სიმწიფის) ფაზაში. მასტიმულირებელ ნაერთთა მაქსიმალური აქტივობა აღინიშნება ფოთლისა და ყვავილედის ზრდის პროცესში (300-225%), ხოლო მომდევნო ფაზებში მათი შემცველობა თანდათანობით იკლებს (180-150%). ნაჩვენებია თუთის მზარდ ფოთლებსა და ყვავილედებში ენდოგენურ აუქსინების აქტივობასა და ლექტინების შემცველობას შორის კორელაციური დამოკიდებულების არსებობა.

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THE EFFECT OF GALACTOSE SPECIFIC MULBERRY LECTIN ON PLANT COLEOPTILES GROWTH ELONGATION

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Abstract

Quality of purification of mulberry seeds of Gal-specific lectin (MNL) promotes sharp stimulation of growth elongation of wheat coleoptiles. Also has to be mentioned that lectin inhibited by carbohydrate loses its ability to influence growth elongation of wheat coleoptiles. Thus participation of MNL in growth elongation processes is realized by sugar-bounded centers.

Key words: mulberry, lectin, coleoptile, growth elongation.

Introduction

Polypeptide signaling is an emerging field in plant biology, particularly in the areas of plant self-defense, fertilization, growth and development. Polypeptides are now considered to be a new class of plant hormones, adding to the list of known plant hormones that include auxins, gibberellins, cytokinins, ethylene, abscisic acid, jasmonic acid and brassinolides [1]. Among those phytohormones mentioned above, auxins are recognized as a major class of plant growth hormones and elicit a wide range of reactivity being responsible for growth and differentiation including cell elongation and cellular differentiation. But the plant science as a whole has much to learn about concrete mechanisms of an action of this plant hormone in the processes of growth and development. Now it becomes more evident that biomolecules containing carbohydrates and with them specifically binding proteins - lectins play key role in control of those physiological processes described above [2]. Therefore, identification of plant cell lectins and study of their function is an actual problem of modern plant physiology.

Based on preliminary works regarding investigation of lectin dynamics in dependence with their content and activity in different organs (root, stem, bud, inflorescent) of mulberry at various stages of their development, supposed possible physiological role of mulberry lectins, which depict their participation in growth and development processes [3].

Material and methods

In this work an influence of Gal-specific lectins of mulberry seeds on plant cells growth elongation has been investigated. Partial purification of the proteins, extracted from mulberry seeds using decreasing activity of K^+-phosphate buffer, was performed by consecutive precipitation in ammonium sulfate (0-90% saturation). Dialyze was performed by chromatography through a Sephadex G-10 column (50 X 2.7 cm) equilibrated with 0.9% NaCl +0.02 M K^+-phosphate buffer (pH 7.4). Toyopearl HW-55 column (1.5X45 cm) equilibrated with the same buffer performed gel-filtration. Following purification of lectins was performed by affinitive chromatography in tris-acryl-galactose column (6x0.8cm). Purity of the lectin was tested by native

electrophoresis. Agglutinating lectinal activity was proven by applying of haemoglutinal test on the trypsinised erythrocytes of the rabbit [4]. Protein concentration was estimated using Lowry method [5]. Biological activity was tested by biotest on growth of clippings of wheat coleoptile [6].

Results and discussions

The quality of purification of Gal-specific lectin (MNL) of mulberry seeds as an individual molecule, by using the method described above is 1097 times higher that of preliminary stages (Table 1). An influence of MNL on growth intensity of wheat coleoptiles elongation zone has been studied in the following series of experiments: 1) MNL (50 mkg/ml); 2) MNL (50 mkg/ml) inhibited by 5 M D-Galactose; 3) 5 M D-Galactose (sugar control); 4) H_2O (pH 7.4) (experiment control). As it is shown on Fig.1, MNL promotes sharp stimulation of growth elongation of wheat coleoptiles. This effect is similar to auxin affection on growth and reaches 250%, which is 2.5 times higher in comparison with the control. It must be mentioned that lectin inhibited by carbohydrate loses its ability to influence growth elongation of wheat coleoptiles. Thus participation of MNL in growth elongation processes is realized by sugar-bounded centers.

Based on these data we can predict participation of Gal-specific lectin of mulberry tree in the process of growth elongation, which is typical for plant cells. This is a new approach in plant physiology, covering study of new plant hormones of polypeptide nature regulating growth development processes. Therefore, this data are important in the investigation of the physiological role of lectins and the molecular mechanisms of growth elongation processes in plants. That is the main purpose of our future investigation.

Table 1.

Stages of purification	Lectin activity (mkg./ml)	Specific activity (ml/mg)	Purification rate at all following stages in comparison with previous stage	Purification rate in comparison with previous stage
Extract	0,72	931	1	1
$(NH_4)_2SO_4$	0,068	14455	16	16
Sephadex G-10	0,029	34133	2,35	37
Affinitive chromatography (GalNAc)	0,00098	1020016	30	1097

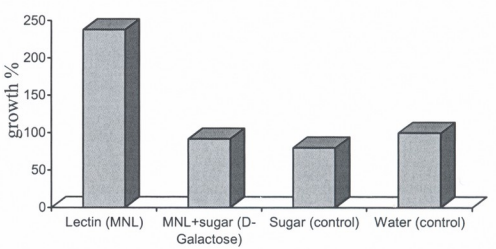

Fig. 1. The effect of galactose specific lectin of mulberry on wheat coleoptiles

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თუთის გალაქტოზა სპეციფიკური ლექტინის გავლენა მცენარეული უჯრედების გაჭიმვით ზრდაზე

ალექსიძე გ., ხურციძე ე., ხურციძე მ.

მცენარეთა ანატომიისა და ფიზიოლოგიის კათედრა, ივ. ჯავახიშვილის სახელობის სახ. თბილისის უნივერსიტეტი

(მიღებულია 28.07.2003)

რეზიუმე

ნაჩვენებია, რომ თუთის თესლებიდან ინდივიდუალური მოლეკულის სახით გასუფთავებული Gal-სპეციფიკური ლექტინი (MNL) იწვევს ხორბლის კოლეოპტილების გაჭიმვით ზრდის მკვეთრ სტიმულირებას. ამასთანავე უნდა აღინიშნოს, რომ ნახშირწყლით (D-გალაქტოზა) დაინჰიბირებული ლექტინი ვეღარ ახდენს გავლენას ხორბლის კოლეოპტილების გაჭიმვით ზრდაზე, რაც იმაზე მიუთითებს, რომ MNL-ის მონაწილეობა გაჭიმვით ზრდის პროცესებში ხორციელდება შაქრის დამკავშირებელი ცენტრების საშუალებით.

100

მედიცინის მეცნიერებათა დოქტორი, პროფესორი, საქართველოს მეცნიერების დამსახურებული მოღვაწე, თბილისის ივ. ჯავახიშვილის სახ. უნივერსიტეტის კათედრის გამგე, გამოჩენილი მორფოლოგი, ალექსანდრე ლეჟავა, კარგად იყო ცნობილი არა მარტო საქართველოში, არამედ მთელ ყოფილ საბჭოთა კავშირში.

მიუხედავად ხანმოკლე სიცოცხლისა მან ადრევე დაიმკვიდრა თვალსაჩინო მკვლევარის სახელი თავისი მეცნიერული შრომებით და აქტიური მოღვაწეობით მეცნიერულ სარბიელზე. 1928 წელს, უნივერსიტეტის დამთავრების შემდეგ აკადემიკოს ალ. ნათიშვილის მიერ მიწვეული იყო ჰისტოლოგიისა და ემბრიოლოგიის კათედრაზე ასისტენტად, შემდეგ დოცენტად. ამავე დროს ის უკვე დამოუკიდებლად კითხულობდა ჰისტოლოგიის კურსს სამედიცინო ინსტიტუტის სტუდენტებისათვის.

ალ. ლეჟავას ცხოვრებაში დიდი როლი ითამაშა სპეციალიზაციის ორმა წელმა ლენინგრადის სამხედრო სამედიცინო აკადემიის ჰისტოლოგიის კათედრაზე ნიკოლოზ ხლოპინის ხელმძღვანელობით. აქვე აკადემიის სამეცნიერო საბჭომ მიანიჭა მას მედიცინის მეცნიერებათა კანდიდატის სამეცნიერო ხარისხი.

თბილისში არ დარჩენილა არც ერთი სათანადო პროფილის სამეცნიერო ინსტიტუტი და უმაღლესი სასწავლებელი სადაც არ ემუშავა და თავისი წვლილი არ შეეტანა ალ. ლეჟავას. თბილისის სახელმწიფო უნივერსიტეტის სამედიცინო ფაკულტეტს, ხოლო სამედიცინო ინსტიტუტის გამოყოფის შემდეგ ამ ინსტიტუტის ჰისტოლოგიის და ემბრიოლოგიის კათედრის ასისტენტი, დოცენტი. თბილისის ვეტერინარულ-ზოოტექნიკური ინსტიტუტის ჰისტოლოგიის კათედრის გამგე. მან ჩამოაყალიბა თბილისის სახელმწიფო უნივერსიტეტში ანატომიის და ჰისტოლოგიის კათედრა და სიცოცხლის ბოლომდე იყო ამ კათედრის გამგე. ომის დროს მან თბილისის ორ ევაკოჰოსპიტალში ჩამოაყალიბა ჰისტოლოგიური ლაბორატორიები, სადაც შეისწავლიდა პერიფერიული ნერვების ცვლილებებს, რომლებიც საფუძვლად უდევს კაუზალგიას. საქართველოს მეცნიერებათა

აკადემიის ალ. ნათიშვილის სახ. ექსპერიმენტული მორფოლოგიის ინსტიტუტის გახსნის დღიდან ის განაგებდა ჰისტოლოგიის განყოფილებას, სადაც განავითარა თავისი კვლევის ძირითადი ინტერესი – გარდამავალი ეპითელის მორფოლოგიის საკითხზე. აქ და თბილისის სახელმწიფო უნივერსიტეტის კათედრაზე მან შემოიკრიბა თავისი მრავალრიცხოვანი თანამშრომლები, რომლებიც სწორედ ამ მიმართულებით კვლევაზე დარაზმა.

მისი სადოქტორო დისერტაციის თემაც იყო გარდამავალი ეპითელი, რომელიც ემყარებოდა მრავალი წლის გამოკვლევებს. სადოქტორო დისერტაცია ალ.ლეჟავამ ლენინგრადში დაიცვა 1950წ., ხოლო 1959წ. გამოაქვეყნა მონოგრაფია.

მეცნიერულ, პედაგოგიურ მუშაობასთან ერთად ალ. ლეჟავა აქტიურ საზოგადოებრივ მოღვაწეობასაც ეწეოდა. იგი იყო მრავალი მეცნიერული ფორუმის მონაწილე და ორგანიზატორი, თბილისის აპშ საზოგადოების თავმჯდომარე, მრავალი დისერტაციის ხელმძღვანელი.

ალექსანდრე ლეჟავას ხსოვნა დიდხანს დარჩება მისი მრავალრიცხოვანი კოლეგების, უშუალო მოწაფეების და იმ სტუდენტების მოგონებაში, ვისაც ჰქონდა ბედნიერება მოესმინა მისი ლექციები.

აკად. ნ. ჯ̌ავახიშვილი

This issue is dedicated to the memory of famous Georgian scientist Alexandre Lezhava. A.Lezhava founded department of anatomy and histology at Tbilisi State University and was head of this department. He also was head of the histology department at Tbilisi Veterinary-Zootechnical Institute, professor of the histology and embriology department of Tbilisi State Medical Institute. From the very first day Natishvili Experimental Morphology Institute opened he had been head of the histology department, where he studied and developed his major scientific interest – morphology of epithelium.

During World War 2 he founded histological laboratories in Tbilisi hospitals where he studied changes of peripherial nerves.

A. Lezhava was head of the Society of anatomy, histology and embriology.

A. Lezhava tutored a lot of students and advised post-graduate students and doctors.

The memory of A. Lezhava will be in the minds of his numerous collegues and students, who had a wonderful opportunity to attend his lectures.

Editorial board

ინსტრუქცია ავტორთათვის

სამეცნიერო წერილებს ჟურნალი ბეჭდავს ინგლისურ ენაზე, ნაშრომს უნდა დაერთოს რეზიუმე ინგლისურ და ქართულ ენაზე. წერილს წინ უნდა უძღოდეს: სამეცნიერო მიმართულება, სათაური, ავტორთა გვარები და მათი სამუშაო დაწესებულების დასახელება.

სამეცნიერო წერილის მოცულობა არ უნდა იყოს 5 გვერდზე ნაკლები და 12 გვერდზე მეტი. წერილი უნდა გაფორმდეს სდანტარტული რუბრიკაციით: შესავალი და მიზნები (introduction), მასალა და მეთოდები (materials and methods), შედეგები და მათი განხილვა (results and discussion), დამოწმებული ლიტერატურა. უკანასკნელი უნდა იყოს დალაგებული ანბანის მიხედვით, ხოლო ტექსტში წყაროების მითითება უნდა ხდებოდეს ფრჩხილებში ჩასმული ავტორის გვარითა და წლით. თითოეულ წერილს უნდა დაერთოს ე.წ. საკვანძო სიტყვათა მოკლე (4-6) სია. ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

ჟურნალის შემთხვევაში:

Carvalho C., Pereira H., Pina C. *Chromosomal G-dark bands determine the spatial organization of centromeric heterochromatin in nucleus.* Mol. Biol. Cell, **12**, 5, 3563-3572, 2001.

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Kuhn T.S. *The structure of scientific revolutions.* Chicago, IL,Chicago Press, 2000. ან

Gentner D., Brush S. *Flowing waters or teeming crowds*. In: Mental Models. D. Gentner (Ed.), Chicago IL., Chicago Press, 865-900, 2001.

ჟურნალში გამოსაქვეყნებელი წერილი წარმოდგენილი უნდა იყოს როგორც ქაღალდზე ამობეჭდილი, ისე კომპიუტერულ დისკეტზე. კომპიუტერზე ტექსტის აკრეფისას ავტორმა უნდა გაითვალისწინოს შემდეგი წესები: ქართული ტექსტისთვის დაუშვებელია ისეთი შრიფტის გამოყენება, რომელიც რუსულ და ქართულ კლავიატურასთანაა დაკავშირებული. ოპტიმალური ფონტებია AcadNusx და AcadMtavr. ინგლისური ტექსტები Times New Roman-ით უნდა იყოს აკრეფილი. შრიფტის ზომა - 12 პუნქტი, ინტერვალი - 1,5. ცხრილებში დასაშვებია უფრო მცირე ზომის შრიფტები. ცხრილები, გრაფიკები და დიაგრამები შესაძლებელია დამზადდეს როგორც Microsoft Word-ში, ისე Excel-ში. მკაფიო გრაფიკები და ფოტოსურათები მიიღება აგრეთვე ოპტიკინაფების (არაელექტრონული) სახითაც. კომპიუტერულ დისკეტზე წარმოდგენილი წერილი მოლიანად ერთი ფაილით უნდა იყოს შენახული, ხოლო ფაილის სახელწოდება წერილის პირველი ავტორის გვარს უნდა ატარებდეს. ცალკე ფაილად შეიძლება ილუსტრაციების წარმოდგენა. დისკეტი უნდა იყოს უხმარი. დისკეტი ავტორს არ უბრუნდება. სურათები, გრაფიკები და სხვა ილუსტრაციები ცალკე ფურცელზე უნდა იყოს წარმოდგენილი. სურათების წარწერები იმავე ფაილის ბოლოს, წერილის ტექსტის შემდეგ უნდა იყოს აკრეფილი. ტექსტი დაკაბადონების გარეშე უნდა აიკრიფოს. წერილი უნდა დაიბეჭდოს A4 ფორმატის ქაღალდზე, ველებით ზედით და ქვედით - 2,5 სმ, მარცხნივ – 3 სმ. და მარჯვნივ – 2სმ.

ჟურნალის ბეჭდვა ავტორთა ხარჯებით ხორციელდება. თანხა რედაქციაში უნდა შემოვიდეს წერილზე დადებითი რეცენზიის მიღებისთანავე. რეცენზენტის მიერ წერილის დაწუნების შემთხვევაში ავტორს უბრუნდება მისი ხელნაწერი, ხოლო დისკეტი ინახება რედაქციაში 1 წლის განმავლობაში.

წერილის რეცენზირება ანონიმურია და ავტორს აქვს უფლება მიიღოს ან არ მიიღოს რეცენზენტის შენიშვნები. უკანასკნელ შემთხვევაში წერილი, დამატებითი გაგზავნება სარედაქციო საბჭოს ერთ-ერთ წევრს. მეორე უარყოფითი დასკვნის შემთხვევაში, წერილი ავტორს დაუბრუნდება.

წერილების ჩაბარება შეიძლება სამუშაო დღეებში, 12-დან 16 საათამდე, შემდეგ მისამართზე: თბილისი, რუსთაველის გამზირი 52, საქართველოს მეცნიერებათა აკადემია, ბიოლოგიის განყოფილება, IV სართული, 429 ოთახი, ტელ: 93-58-92, სწავლული მდივანი – მაია გრიგოლავა.