The Inhibition of the Genotoxic Effects of Environmental Pollutants and the Aging processes by Plant Antimutagens

Urkhan ALEKPEROV, Rena GULIEVA
Institute of Genetics, 155 prospect Azadliq, 370106, Baku, Republic of Azerbaijan
Ramiz ALEKPEROV
Azerbaijan Medical University, 23 Bakikhanov street, 370022, Republic of Azerbaijan

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Abstract: We investigated the effects of bioactive compounds obtained from fruits of Diospyros kaki L., Cydonia oblonga Mill. and roots of Glycyrrhiza glabra L., as well as their action in a complex mixture on the mutability in marrow cell chromosomes induced by genotoxicants (X-rays, N-Methyl-N-Nitrosurea, Cyclophosphamide) and aging. The plant products tested separately and in a complex mixture showed an ability to decrease the frequency of induced mutations in experimental animals. Antimutagenic properties of the complex mixture turned out to be considerably higher than its components separately. More antimutagenic activity of the mixture was revealed when mutagenesis was the result of X-rays and aging. The administration of this mixture was included in a therapy program of a group of volunteers with a high level of chromosome aberrations in lymphocytes, caused by acute pesticide intoxication. Administration of the mixed antimutagens decreased the level of aberrations to control values.

Key Words: antimutagen, anticarcinogen, environmental genotoxicants, aging, acute intoxication, therapy of genetic complications.

Çevre Kirleticilerinin ve Yaşlanmanın Genotoksik Etkilerinin Bitki Antimutagen’lerince Önlenmesi


Akut pestesid zehirlenmesi sonucu lenfositlerinde iki derecede kromozom bozukluğu olan bir grup görülenlere bu bitki mahsul karışımlar terapotik bir program dalleme verilmesi, kromozom bozuklarını kontrol düzeyine indirdi.

Anahtar Kelimeler: Antimutagen, antikarsinogen, çevre genetoksikanları, yaşlanma, akut zehirlenme, genetik komplikasyonların tedavisi.
Introduction

Internal and external factors, including environmental pollutants, induce various kinds of genetic damage. These lead to disorders of both the generational and regulational functions of the genetic apparatus, that cause many pathologies and disease in plants, animals and man (1-3). The propylaxis for the genetic consequences of the action of environmental factors can be done at the technological, component and compensational levels (4). Antimutagenesis based on increasing the stability of the biological systems is an element of the compensational approach and is considered one of the most feasible ways for inhibiting the negative effects of environmental genotoxicants, including carcinogens (5, 6). Recently an investigation was conducted in order to study the possibility of simultaneous antimutagenic correction of the genome in generational and regulational disfunctions (7).

Today a large number of AMs of plant origins are known (8-10). The mechanisms by which AMs decrease the level of mutations are different due to specificity to influence certain stages of the mutation production processes. Antimutagenic effects may result from the chemical and enzymatic inactivation of genotoxicants (11) as well as from inhibition of metabolic activation of promutagens or inactivation of activated genotoxicants (12). It has been shown that these compounds can also correct the mutation process by effects on DNA replication and repair (13). Some plant AMs have exhibited universal properties of the effects on mutation production due to simultaneous influence on the different stages of this process (14, 15), but the level capable of interacting with each other and of entering the metabolic processes. Therefore the complex of environmental pollutants provokes genetic damage due to the simultaneous disorder of various processes guaranteeing normal genome functioning. Thus, it would seem necessary to create a new class of inhibitors of mutability-the AM mixtures, components of which are capable of simultaneously influencing different stages of the mutation process. This report presents the results of a study investigating such a class of AMs.

Materials and Methods

Chemicals and reagents

As inducers of mutations the following chemicals were tested: N-methyl-N-nitrosurea (MNU), Radian Corporation (Austin, TX); Cyclophosphamide (CP) commercially obtained from Saransk, Russia, (Pharmacological Plant). Reagents for lymphocyte culture: Phytohemagglutinin (Wellcome); Giemsa (Merck); Colchicine (Fluka AG); Trypsin (Varion, Russia); bovine serum (Moscow, Russia).

Test substances

As inhibitors of mutations, extracts from the roots of Glycyrrhiza glabra L. (GG) and the fruits of the Diospyros kaki L. (DK) and Cydonia Oblonga Mill. (CO) were studied. The extracts were prepared by homogenizing the plants in 70° ethanol cold extraction in a soxhlet apparatus followed by vacuum distillation of solvent and lyophilization of the final product. The preparation from GG was tested at 0.1 mg/100 g body weight concentration whereas those from DK and
CO were used at 0.5 mg/100 g body weight concentration. The antimutagenic effects of the preparations were analyzed both separately and in a mixture, containing 0.23 mg of DK preparation, 0.23 mg of CO preparation and 0.04 mg of GG preparation. The mixture was tested at 0.5 mg/100 g body weight concentration.

Animals: Treatment, Chromosome Preparations

1.5-2 month-old CBA male mice weighing 16-20 g and 26-32 month-old Wistar male rats weighing 250-320 g were obtained from the Institute of the Preventive Medicine of the Ministry of Health, Republic of Azerbaijan and Stolbovaya Breeding Station of the Russian Academy of Medical Sciences. Both the experimental and control groups of animals were housed six per cage (97x97 with wood bedding), in an environment maintained at 23±2°C, relative humidity 50±5% and with 12 h light/dark. The animals were fed a standard diet (chemical content in percentages: Raw protein 22.8% Raw fat 4.9%; carbohydrates 51.2%, cellulose 1.96%; the cellulose deficit was compensated with the addition of 20 g grassy forur per 1 kg of feed; and tap water ad libitum).

Experimental groups (CBA mice) were given test extracts at 0.5 mg/100 g body weight concentration once a day for 7 days. On the 8th day one group of animals was irradiated at 325 GRy (X-ray apparatus RUM-11 (Russia), distance 40 cm 130 gy/min, 2.5 min). The other three groups of animals on the 8th day were orally administrated the test chemical once per day: NMU (second group), at 5 mg/100 g body weight doses and CP (third group) at 10 mg/100 g body weight doses. Untreated control animals were intact for the whole experimental period, receiving sterile distilled water and mutagen control animals received sterile distilled water for 7 days and on the 8th day were treated with mutagens. The old animals (26-32-month-old Wistar male rats) were orally administrated test extracts for 30 days at 0.5 mg/100 g body weight once a day. The animals were decapitated, mice on the 9th day and rats on the 31st day. Isolated thigh-bones were fixed in absolute alcohol and acetic acid (3:1). For chromosome preparations isolated marrow was stained with acetoarcein (Merck). The frequency of chromosome aberrations (CA) analyzed in the squash preparations was determined. The method used allowed for analysis of single and double fragments of chromosomes, chromatid and chromosome dicentrics at anaphase and early telophase myelokaryocytes under the microscope (Zeiss, Germany).

Humans: Treatment, Chromosome preparations

Twelve patients at the Azerbaijan State Medical University Toxicology Center (6 mean and 6 women) suffering from acute oral intoxication by the organophosphate pesticide methylparathione were divided into two groups each consisting of 3 men and 3 women and
were kept under observation. The patients were of different ages ranging from 16 to 32 and had no previous occupational contact with pesticides. The control group consisted of 10 volunteers (5 men and 5 women, aged 25-30), all non-smoking and non-drug-users. The analysis of CA in peripheral blood lymphocytes was made one day prior to their culture preparations and the analysis of CA in the metaphase cells were made according to the standard methods (17). 10 ml of blood was taken and lymphocytes were separated and cultivated in 199 IGLA nutritional culture mixed with native bovine serum in a 3:1 ratio. Before cultivation, lymphocyte mitogenesis was stimulated with phytohemagglutinin at a dose of 20 mg. After 21 hours of cultivation, colchicine was added to the medium at 0.5 mg/ml for two hours. After the separation of lymphocytes by centrifugation they were treated with hypotonic solution of pure CaCl for easy membrane destruction for 30 minutes and then the cells were fixed in a solution of acetic acid and absolute alcohol in a 1:3 ratio. The preparations were stained with Giemsa. The AM mixture was orally administered once a day at 15 g doses to every patient for 7 days. The aberrations in the human cells were scored as breaks only if the fragments were clearly separated and disoriented from the main chromatid.

Statistical Analysis

Six animals for each experimental variant were used and for anaphase analysis a minimum of 150 cells from each animal were examined (in total a minimum of 900 cells per experimental variant). The data was analyzed with Student's t-test and presented with standard errors. Antimutagenic efficiency was determined as the proportion of the difference between initial and modified levels of mutability to the initial one (in %). A similar method was used for the metaphase analysis: a minimum of 200 cells per patient were analyzed.

Results

The effects of the test substances on the prevention of CA in mouse marrow cells are given in Table 1. All substances inhibited mutagenesis with all genotoxic agents. The highest modifying effect of bioactive compounds was by DK inhibiting mutations induced by X-rays (47%) and CP (50%), whereas GG extract appeared to be more effective in the inhibition of mutability induced by NMU (46%). Similarly, bioactive compounds from CO exhibited the highest antimutagenic efficiency while inhibiting mutability caused by aging (50%) (Table 2). The preparations possessed not the highest but stable antimutagenic efficiency regarding all the mutation inducers used in our experiment also (Table 1).
The effects of the test substances on spontaneous CAs in aged RATs are given in Table 2. The highest efficiency of composition was achieved in the inhibition of genetic disorders caused by aging. Statistically significant changes in the aberrations spectrum were not observed.

The comparative study of the influence of the antimutagenic mixture and its separate compounds on the level of the mutability induced by different agents demonstrated that the antimutagenic effectiveness of the mixture appeared to be considerably higher than those of its components tested separately. Such a synergistic inhibitory effect was achieved for all the mutagens used in our experiments, but was mostly expressed while inhibiting the mutability induced by X-rays and aging. As shown, the antimutagenic efficiency of the mixture exceeded that of its components tested separately by 20–40%. In addition, the mixed antimutagens’ efficiency was much higher than the maximum efficiency noted for each plant extract tested separately (Table 1). The efficiency of the antimutagen mixture was tested on volunteers suffering from acute pesticide intoxication. The results obtained are presented in Table 3. A high level of chromosome aberrations in peripheral blood lymphocytes in all patients was found. In the group of patients given the antimutagenic composition orally, a lower frequency of chromosome aberrations was found. The analysis revealed that the spectrum of aberrations was characterized mainly by changes in the number of single and double fragments.

<table>
<thead>
<tr>
<th>Test substance</th>
<th>X-rays</th>
<th>NMU</th>
<th>CP</th>
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<tr>
<td>None</td>
<td>22.76±1.27</td>
<td>18.76±1.15</td>
<td>12.78±1.02</td>
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<tr>
<td>DK</td>
<td>11.99±1.01**</td>
<td>12.07±1.02*</td>
<td>6.35±0.76*</td>
</tr>
<tr>
<td>CO</td>
<td>13.73±1.01*</td>
<td>11.34±0.95*</td>
<td>7.15±0.62*</td>
</tr>
<tr>
<td>Mixture</td>
<td>6.60±0.67*</td>
<td>7.61±0.7*</td>
<td>3.96±0.6*</td>
</tr>
</tbody>
</table>

Table 1. Aberration analysis in mice marrow cells after exposure to the plant antimutagens and different mutagens in comparison with the unaltered control (1.88±0.39% of aberrant cells).

*P<0.01  **P<0.001

<table>
<thead>
<tr>
<th>Variants of the experiment</th>
<th>Number of cells</th>
<th>Aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.248</td>
<td>111</td>
</tr>
<tr>
<td>DK</td>
<td>1.238</td>
<td>65</td>
</tr>
<tr>
<td>CO</td>
<td>1.490</td>
<td>65</td>
</tr>
<tr>
<td>GG</td>
<td>1.488</td>
<td>95</td>
</tr>
<tr>
<td>Mixture</td>
<td>1.395</td>
<td>32</td>
</tr>
</tbody>
</table>

Table 2. Chromosomes aberrations in marrow cells of old rats after exposure to the plant antimutagens.

*P<0.01  **P<0.001
Discussion

One of the most important characteristics of the comparison of AMs, is their universality regarding inhibition of mutability induced by different stressing agents (4). Today a number of AMs are known and characterized by the high capacity for correcting mutation production simultaneously at different stages. Such a property has been revealed in natural compounds, both pure substances and extracts. It has been stated, for example, that some vegetable extracts (a sum of natural compounds) and alpha tocopherol (pure substance) are capable of affecting the process of mutation production at the stages prior to the occurrence of DNA damage as well as the following processes of replication and reparation (13). At the same time there is a specificity of AM activity that may result in insufficient efficiency of mutation inhibition based on different types of genome disorders. This was confirmed while examining the antimutagenic activity of preparations obtained from DK, CO and GG. It is well known that extracts these plants are characterized by the presence of the following substance having antimutagenic properties: the complex of polyphenol substances (catechine, leucoantociane); flavonoids, including liqiritine, liqintingenin, glabrozide, terpenoids (spanon, carotenoids); mono-, di- polysaccharide (glucose, fructose, saccharose), organic acids (malic, citric); lipidsteroid complex containing 3 starurated, 4 monosaturated and 3 polysaturated fatty acids); aminoacids (gistidine, valine, leucine, glutamine acid, proline, arginine, asparagine); oxidation enzymes (ascorbinaze, catalase, polyphenoloxidase, peroxidase); digestive enzymes (amylase, invertase and pectolitic complex), vitamins (C, B, D), micro and macroelements (10).

<table>
<thead>
<tr>
<th>G</th>
<th>Number of cells</th>
<th>Aberrations</th>
<th>%</th>
<th>B</th>
<th>F</th>
<th>CE</th>
<th>AF</th>
<th>TR</th>
<th>Others</th>
<th>Gaps</th>
<th>CWG</th>
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<tbody>
<tr>
<td>GC</td>
<td>2017</td>
<td>1.09</td>
<td>1.28±0.25</td>
<td>17</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>64</td>
<td>59</td>
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<tr>
<td>GA</td>
<td>1310</td>
<td>1.18</td>
<td>2.9±0.46</td>
<td>23</td>
<td>14</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>79</td>
<td>63</td>
</tr>
<tr>
<td>GB</td>
<td>1276</td>
<td>1.10</td>
<td>1.56±0.34</td>
<td>12</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>37</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 3. Analysis of chromosome aberrations in culture of peripheral blood lymphocytes of patients with acute intoxication

GA.-group of patients with acute intoxication; GB.-group of patients with acute intoxication-antimutagen treatment; GC.-healthy control group.

B-break; F-fragment; CE-chromatid exchange; AF-acentric fargment; TR+QR-triradials and quandriadials

CWG-cells with gaps.
As the data show, the formulation of a new AM of three plant preparations results in a significant increase in the efficiency of inhibition. It is important to note that there is not a simple summing up of the maximum efficiency of the components. The effect of the AM mixture exceeded the maximum positive effect which was revealed while testing each plant preparation separately. Such a sinergistic effect was observed while inhibiting the genotoxic activity of all the genotoxic agents tested. Mostly it was shown by a decrease in the genetic damage caused by aging. Since one of the factors determining premature aging is the accumulation of the genome damages caused by the combined effects of environmental pollutants (14), a new practical use of compositional antimutagens in genoprotection becomes possible. From a practical point of view, the possibility of reducing the genotoxic effects of acute intoxication by administration of compositional antimutagens is also very high.

From the results we can conclude that the use of this class of AMs appears to open up the possibility of increasing the efficiency of antimutagenic protection cells’ genetic structures from genotoxic effects. Further studies in this field and in particular the creation of new complexes are of great importance.

References


