EVALUATION OF GENOTOXIC EFFECTS OF IPRONIDAZOL (GASTROGAL 10®) IN CULTURES OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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(Received 25. May 2002)

In this work, we examined the genotoxic effect of the antibiotic preparation ipronidazol (Gastrogal 10). An experiment was performed in vitro on human peripheral blood lymphocytes using the chromosome aberration and sister chromatid exchange tests. Clastogenic effects of ipronidazol were examined at three experimental concentrations (25 µg/ml, 50 µg/ml i 100 µg/ml) in eight experimental cycles. The results demonstrate that Gastrogal 10 has the ability to change the human lymphocyte karyotype, i.e. it induces numerical aberrations (aneuploidies and polyploidies), as well as chromosome gaps and breaks. Moreover, Gastrogal 10 induces a significant increase of sister chromatid exchange in human lymphocytes. The obtained results demonstrate that the examined preparation exhibits a certain genotoxic potential.

Key words: chromosome aberrations, Gastrogal 10, ipronidazol,

SCE

INTRODUCTION

The application of genotoxic agents and their possible accumulation in living systems cause irreversible consequences such as lethal mutations, frequent gamete loss, embryo mortality, congenital malformations, changes of genetic variability in populations and increased incidence of carcinoma (Anderson et al., 1994). Therefore genotoxicological studies should be supplemented by research on the pharmacokinetics of toxic compounds, in order to broaden our knowledge about the role of genotoxic agents in such complex processes as mutagenesis and carcinogenesis.

Environmental mutagenesis has special features, because various compounds can increase gene mutation frequency, despite the possibility of a high level of survival in exposed populations. (Legator, 1994; Muller and Kasper, 2000). Namely, mutations can be induced even at concentrations that exhibit very low cytotoxicity (Kirkland, 1998; Kovaikovicova et al. 2001). Since the genotoxic agents comprise a large number of pharmaceutical preparations that can express the above mentioned properties, it is important to evaluate their potential genotoxicity (FAO/WHO, 1995). Literature data on the genotoxic potential of ipronidazol are scarce. Voogd et al. (1977) demonstrated that ipronidazol...
(fluctuation test) increases mutation frequency during the following three to four generations in a prokaryotic system. In addition, ipronidazol influences redox processes in heart mitochondria. Low concentrations of ipronidazol decrease redox processes by 20%, whereas higher concentrations cause a more profound effect - 60% a decrease. Metabolic changes under the influence of ipronidazol comprise a decrease of FMN protein level and lowered intensity of ADP phosphorylation in the presence of gluco-malate substrate (Aicardi and Solani, 1982).

According to the World Health Organisation (WHO, 1998), ipronidazol exhibits carcinogenic properties. Therefore restricted administration of ipronidazol is recommended. Since there is a general tendency to use pharmacological preparations with lower toxicity and genotoxicity, the aim of the present investigation was to test Gastrogal 10, a widely used preparation in veterinary medicine, for genotoxicity under in vitro conditions.

MATERIALS AND METHODS

Test substance. Gastrogal 10 (ICN Galenika, Beograd) contains 100 mg of ipronidazol per ml of solution.

Treatment. Freshly prepared dilutions were added to cultivation vials to obtain final concentrations of 25 μg/ml, 50 μg/ml and 100 μg/ml at the beginning of incubation. Control (untreated) cultures were set up in each experimental cycle. There were eight experimental cycles.

In vitro chromosome aberration test. Metaphase spreads were obtained according to the method of Evans and O’Riordan (1976) as modified by Zimonjić et al. (1990). Briefly, human lymphocytes cultures were prepared heparinised whole blood of healthy men under 35 years of age, added to Parker 199 media (Tordak, Belgrade), containing 30% heat inactivated calf sera (VZ, Subotica) and 0.04 mg/ml of phytohaemagglutinin (Murex, Dartford, England). The cultivation vials were incubated for 72 h at 37°C. Two hours before harvesting colchicine (Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 0.5 μg/ml. After the hypotonic treatment (0.075 M KCl) followed by three repetitive cycles of fixation in methanol/acetic acid solution (3: 1, v/v), centrifugation, and resuspension, the cell suspension was dropped on chilled grease-free microscopic slides, air dried and stained in 10% Giemsa (Kemika, Zagreb, Croatia) solution.

Sister-chromatid exchange test. Cultures were set up in the same way except that 5-bromo-2'-deoxyuridine (BrdUrd, Sigma Chemical Co., St. Louis, MO) was added at the beginning of incubation at a final concentration of 25 μM. The visualisation of differentially stained chromatids was achieved by the method of Perry and Evans (1975), slightly modified by Zimonjić et al. (1990).

Statistical analysis. Comparison of the significance between treated and untreated cultures was performed by Student’s t-test.
RESULTS

The results obtained in this experiment demonstrate that ipronidazol (Gastrogal 10) induced numerical aberrations (aneuploidies and polyploidies) and structural lesions (chromatid gaps and breaks) at all experimental concentrations. (Table 1, Figure 1).

Table 1. Cytogenetic parameters in control and experimental cultures of human peripheral blood lymphocytes treated with increasing doses of Gastrogal 10

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>Gastrogal 10 25 µg/ml</th>
<th>Gastrogal 10 50 µg/ml</th>
<th>Gastrogal 10 100 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± SD  %</td>
<td>X ± SD  %</td>
<td>X ± SD  %</td>
<td>X ± SD  %</td>
</tr>
<tr>
<td>Aneuploidy</td>
<td>1.63 ± 0.82</td>
<td>14.75 ± 7.38</td>
<td>21.88 ± 10.94</td>
<td>31.75 ± 15.88</td>
</tr>
<tr>
<td>Polyplody</td>
<td>0</td>
<td>2.13 ± 1.07</td>
<td>5.5 ± 2.75</td>
<td>7.87 ± 3.94</td>
</tr>
<tr>
<td>Breaks</td>
<td>0.63 ± 0.32</td>
<td>6 ± 3</td>
<td>13.62 ± 6.81</td>
<td>18.25 ± 9.13</td>
</tr>
<tr>
<td>All cyto genetic changes</td>
<td>4.26 ± 2.14</td>
<td>34.51 ± 17.27</td>
<td>60.13 ± 30.67</td>
<td>82.12 ± 41.08</td>
</tr>
</tbody>
</table>

There was positive dose-dependence, in that the lowest concentration (25 µg/ml) caused aberrations in 17.27% cells, whereas the concentration of 50 µg/ml induced cytogenetically detectable changes in 30.67% of the examined mitoses. Finally, the highest concentration (100 µg/ml) caused cytogenetic changes in 41.08% cells. Statistical analysis by Student's t-test revealed significant differences between control and treated cultures. Moreover, the same level of statistical significance existed between cultures treated with different concentrations of Gastrogal 10 (Table 2).

In addition to the chromosome aberration assay, we investigated possible effects on DNA using a sister chromatid exchange (SCE) test in vitro. The results are shown in Table 3 and Figure 2. The mean value of SCE per cell frequency in the control group amounted to 1.07 ± 0.04. Treatment with Gastrogal 10 caused a dose-dependent increase of SCE frequency per cell. Thus, in cultures treated with 25 g/ml the mean value was 4.40 ± 0.15. The intermediate concentration (50 g/ml) and the highest concentration applied (100 µg/ml) caused more profound elevation of SCE frequency per cell with mean values of 7.47 ± 0.30 and 9.97 ± 0.17, respectively. The level of statistical significance in relation to the control group was relatively high (p<0.01) even after treatment with the lowest concentration of Gastrogal 10, whereas at higher concentrations statistical significance was more profound (p<0.001).
Figure 1. Percentage values of cytogenetic parameters in control and experimental cultures of human peripheral blood lymphocytes treated with increasing doses of Gastrogal 10.

Table 2. Level of statistical significance between controls and Gastrogal 10-treated groups for total cytogenetic parameters monitored.

<table>
<thead>
<tr>
<th></th>
<th>X ± SD</th>
<th>Gastrogal 10 25 µg/ml</th>
<th></th>
<th>Gastrogal 10 50 µg/ml</th>
<th></th>
<th>Gastrogal 10 100 µg/ml</th>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>4.26 ± 1.83</td>
<td>0.94</td>
<td>***</td>
<td>0.85</td>
<td>***</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Gastrogal 10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 µg/ml</td>
<td>34.51 ± 1.92</td>
<td>32.15</td>
<td></td>
<td>49.88</td>
<td></td>
<td>90.44</td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>60.13 ± 2.58</td>
<td>22.47</td>
<td></td>
<td>52.98</td>
<td></td>
<td>19.63</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>82.12 ± 1.55</td>
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</tbody>
</table>
Table 3. Frequency of sister chromatid exchanges (SCEs) in control and experimental cultures of human peripheral blood lymphocytes treated with increasing doses of Gastrogal 10

<table>
<thead>
<tr>
<th>Treatments</th>
<th>SCE range</th>
<th>Mean value of SCE/cell</th>
<th>SD</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0-3</td>
<td>1.07</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Gastrogal 10 25 μg/ml</td>
<td>1-8</td>
<td>4.40</td>
<td>0.15</td>
<td>4.26**</td>
</tr>
<tr>
<td>Gastrogal 10 50 μg/ml</td>
<td>3-12</td>
<td>7.47</td>
<td>0.30</td>
<td>42.49***</td>
</tr>
<tr>
<td>Gastrogal 10 100 μg/ml</td>
<td>4-15</td>
<td>9.77</td>
<td>0.17</td>
<td>93.45***</td>
</tr>
</tbody>
</table>

**p<0.01  
***p<0.001

Figure 2. Average values of SCE in control and experimental cultures of human peripheral blood lymphocytes treated with increasing doses of Gastrogal 10
DISCUSSION

Analysis of metaphase spreads obtained from cultures treated with various concentrations of Gastrogal 10, revealed that all concentrations applied in this investigation induced numerical aberrations (aneuploidies and polyplloidies) and structural alterations visible as chromatid gaps and breaks. The appearance of aneuploid cells can be explained by changes or dysfunction of microtubules (Clements and Todd 1981). Therefore, we assume that Gastrogal 10 may interfere with the formation or normal function of microtubules, thereby inducing aneuploidies. Moreover, the tested antibiotic preparation was able to induce polyplloidies, thereby reflecting the genotoxic potential of ipronidazol (Marković, 1999). In addition to numerical aberrations, all applied concentrations induced structural changes (gaps and breaks) on human lymphocyte chromosomes. These changes result either from primary DNA lesions or damage to the chromosomal protein substrate (Delić 1997; Stanimirović et al. 1998; Marković, 1999). It is evident that the percentage of overall cytogenetic changes increased with the elevation of Gastrogal 10 concentration in the culture media. Although Gastrogal 10 had the ability to induce chromosome aberrations at all concentrations applied, the close dose-dependence clearly demonstrates its genotoxic potential.

Abundant literature data indicate that the level of spontaneous SCE frequency per cell varies between 1.4 and 4.5, depending on the cell type, culture conditions, age of donors and other circumstances (Marković et al., 2000). Thus, the mean value of spontaneous SCE frequency per cell is relatively low and according to some authors is approximately 1.1. The analysis of SCE frequency per cell on cultured human lymphocytes treated with Gastrogal 10, revealed that mean value of SCE increased steadily from 4.40 ± 0.15 at the concentration of 25g/ml to 9.97 ± 0.17 at the highest concentration (100 g/ml). While the SCE frequency per cell induced by the lowest concentration of Gastrogal 10 is comparable to the spontaneous level observed in some laboratories, the higher concentrations induced a highly significant increase of SCE frequency compared to the control values of spontaneous SCE occurrence. The ability of Gastrogal 10 to increase SCE frequency per cell possibly results primary damage at the level of DNA (Oikawa et al., 1983). It is conceivable that ipronizadol directly or indirectly interacts with nucleic acids and/or facilitates the appearance of DNA damage (Bila and Kren, 1992).

On the basis of the obtained results it can be concluded that the antibiotic preparation Gastrogal 10 has the ability to change the karyotype of human lymphocytes. Gastrogal 10 has genotoxic potential that can be detected via induction of structural and numerical chromosomal aberrations. The SCE per cell demonstrates that tested concentrations exhibit various level of genotoxicity. Namely, the lowest concentration of Gastrogal 10 induces SCE per cell frequency comparable to spontaneous changes, whereas higher concentrations induce a strong genotoxic effect exhibited by the very high level of SCE.

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REFERENCES


EVALUACIJA GENOTOKSIČNOG EFEKTA IPRONIDAZOLA (GASTROGALA 10®) U KULTURI HUMANIH LIMFOCITA PERIFERNIH KRVI

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SADRŽAJ

U ovom radu su izneti rezultati ispitivanja genotoksičnih efekata antibiotičkog preparata Gastrogala 10 čija je aktivna supstanca ipronidazol. Eksperiment in vitro je obavljena na humanim limfocitima, periferne krvi, primenom testa indukcije hromozomskih aberracija u kulturi ćelija, kao i testa ispitivanja
oštećenja na nivou molekula DNK, praćenjem razmene sestrinskih hromatida (SCE). Ispitivanje klastogenog efekta odgovarajućih doza Gastrogala 10 (25 µg/ml, 50 µg/ml i 100 µg/ml) obavljeno je kroz 8 eksperimentalnih ciklusa. Rezultati istraživanja in vitro pokazuju da ispitivani antibiotski preparat ima sposobnost promene kariotipa limfocita čoveka i indukcije numeričkih hromozomskih aberacija tipa aneuploidija i poliploidija i strukturnih hromozomskih promena tipa lezija i prekida. Gastrogal 10 indukuje značajno povećanje razmene sestrinskih hromatida u humanim limfocitima. Dobijeni eksperimentalni rezultati, ukazuju da ispitivani antibiotski preparat Gastrogal 10 ima određeni genotoksični potencijal.