Cytogenetic Effects of Benzene on Human Blood Cells

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Abstract
The study aims to investigate the cytogenetic effect of benzene on human blood cells in vitro using chromosomes abnormalities and mitotic index test. Different concentrations of benzene were added to human blood culture at 24 hour, then cells were arrested at metaphase to detect chromosomes malformations and its proliferation, the result show that benzene causes increased in mitotic index level and different aberrations in chromosomes which increased with benzene concentrations.

Keywords: Benzene, Chromosomes abnormalities, Mitotic index.

1. Introduction
Benzene is an important pollutant compound, present in both occupational and general environment. Chronic exposure to high concentrations of benzene in human is associated with an increased incidence of myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) (Baudouin et al., 2002). It is well known that individuals occupationally exposed to benzene are at a much higher risk of developing leukemia than the normal population (Carere et al., 1998). The absorption, distribution, metabolism and excretion of benzene have been intensively investigated in several experimental animal species and in humans. Benzene is readily absorbed from oral and inhalation exposures. Dermal absorption is also rapid; however, quantitatively, dermal absorption is very low due to rapid evaporation from skin. Benzene is rapidly distributed throughout the body after exposure by all routes, and accumulation in fatty tissues is observed (ATSDR. 2007).

In studies of occupational exposure, benzene was found to cause chromosome changes at concentrations that induced blood dyscrasias (Weisel, 2010). At concentrations below (31 ppm), workers exposed for 10 to 26 years had significantly more chromosome breaks and gaps in peripheral lymphocytes than found in controls, and 31 of the 33 workers had no other evidence of clinical or hematological effects (Rappaport, et al., 2009).

At exposure levels of less than (10 ppm) over one month to 26 years, workers also had a significantly higher number of chromosomal aberrations in peripheral lymphocytes than controls (Khalade, et al., 2010). Benzene has been also implicated as an environmental risk factor in leukemia and other hematological diseases. The main sources of environmental exposure to benzene are road traffic exhaust (Zhang, et al., 2006) and volatile organic compounds; this means urban air pollution in general (Bi, Y. et al., 2009). Lifestyle factors, such as cigarette smoking, can contribute to exposure (Eastmond et al., 1994). The soil obtained from oil production facilities and coastal refineries is also highly contaminated by benzene (Eastmond et al., 2001).

2. Materials and methods

2.1. Blood samples: Blood samples were collected from 25 healthy (male and female) in Age (20±5 years), that was not directly exposed to benzene and they are smoker.

2.2. Blood culture: Blood planting according to (Chen et al., 1994), then plant of blood divided in to five gropes every gropes had 5 replicates.

2.3. Treatment: after 24 hours from incubating, 300 µl of different benzene Concentrations was added to blood culture.

1- First group (2.5 × 10⁻⁵) molar
2-Second group (5× 10⁻⁵) molar
3-Third group (10× 10⁻⁵) molar
4-Fourth group (20× 10⁻⁵) molar
5-Fifth group without any addition of benzene and this group is the negative control. Then cytogenetic tests were performed according to (Clare et al., 1984).
3. Results
Treatment human blood cells with different concentrations of benzene causes increased in mitotic index as shown in table (1), also causes different chromosomal abnormalities as shown in table (2) and figure (1, 2).

4. Discussion
The association between benzene exposure and the appearance of structural and numerical chromosomal aberrations in human lymphocytes suggests that benzene may be considered as a human clastogen. In animal studies, benzene induced cytogenetic effects, including chromosome and chromatid aberrations, sister chromatid exchanges, and micronuclei (Clare et al., 1984, Liu et al., 2000, Liu et al., 2003). There is some evidence that benzene can induce chromosomal abnormalities in mammalian cell cultures (Marcon et al., 1999). Metabolites of benzene (hydroquinone, catechol, diol epoxides and trihydroxybenzene) induced sister chromatid exchanges in V79 cells (Zhang, L. et al., 2010) several metabolites, including muconaldehyde, have induced micronuclei in cell cultures (Ji, Z. et al., 2010).

Benzene can induce structural and numerical chromosome aberrations, sister chromatid exchanges and micronuclei by various routes of exposure (North, M. et al., 2009). Most studies were performed with fairly high concentrations, but (Badham et al., 2010) detected sister chromatid exchanges in peripheral lymphocytes and micronuclei in the bone marrow of rats at 9.6 and 3.2 mg/m3, respectively (Badham, H.J. et al., 2010). were able to detect chromosome aberrations in lung macrophages after prolonged exposure (6 weeks) at concentrations as low as 0.32 mg/m3, and in lymphocytes from the spleen of mice at 0.13 mg/m3 (Shuga et al., 2010). However, there was no dose–response relationship in the latter study, as the highest exposure (3.2 mg/m3) produced fewer aberrations than the middle exposure (32 mg/m3).

The chromosomal effects in these studies are evident at concentrations of around 320 mg/m3 (100 ppm) or higher, but in some studies effects were reported in workers chronically exposed to levels of around 32 mg/m3 (10 ppm) (Gillis et al., 2007). Sarma et al. (2011) reported that the frequency of chromosome aberrations decreased when exposure levels decreased from 3–69 mg/m3 to 1–18 mg/m3 (Sarma et al., 2011). In the study by Martínez-Velazquez, M. et al. (2006) a decrease in sister chromatid exchanges but not in chromosomal aberrations was noted in a group of female workers when examined with a 5-year interval during which the mean benzene concentration had decreased from 26 to 16 mg/m3 (Martínez-Velazquez, M. et al., 2006). Smoking did not influence the results (Sasiadek et al., 1998).

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References


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Zhang, Y. et al. (2006) Chromatin structural elements and chromosomal translocations in leukemia. DNA Repair (Amst.), 5, 1282–1297

Figure (1): Percentage of chromosomes abnormalities in human blood cells that treated by different concentration of benzene. (1) NC, (2) $2.5 \times 10^{-5}$, (3) $5 \times 10^{-5}$, (4) $10 \times 10^{-5}$, (5) $20 \times 10^{-5}$
Figure (2) chromosomes abnormalities in human blood cells treated by (10× 10⁻⁵ and 20× 10⁻⁵ molar) of benzene.

Table (1) effect of different concentrations of benzene on cell mitotic index

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>8.64</td>
</tr>
<tr>
<td>2.5 × 10⁻⁵</td>
<td>10.6</td>
</tr>
<tr>
<td>5 × 10⁻⁵</td>
<td>10.6</td>
</tr>
<tr>
<td>10 × 10⁻⁵</td>
<td>10.6</td>
</tr>
<tr>
<td>20 × 10⁻⁵</td>
<td>13.45</td>
</tr>
</tbody>
</table>

Table (2) Differential chromosomal abnormalities in human blood cell that treated by different concentration of benzene.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Polycentric chrom.</th>
<th>Minute Chrom.</th>
<th>Chromosome stickiness</th>
<th>Ring Chrom.</th>
<th>End to end association</th>
<th>Deletion of chromatid</th>
<th>Bizarre configuration</th>
<th>Deletion</th>
<th>Centromere association</th>
<th>Aneuploidy</th>
<th>Hyperdiploidy</th>
<th>Chromatid break</th>
<th>Chrom. break</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. control</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2.5 × 10⁻⁵</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>5 × 10⁻⁵</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>10 × 10⁻⁵</td>
<td>0.1</td>
<td>0.1</td>
<td>4</td>
<td>2</td>
<td>0.5</td>
<td>3.7</td>
<td>5.3</td>
<td>4</td>
<td>2.5</td>
<td>1</td>
<td>0.6</td>
<td>3.2</td>
<td>2</td>
</tr>
<tr>
<td>20 × 10⁻⁵</td>
<td>0.1</td>
<td>0.2</td>
<td>2.3</td>
<td>3</td>
<td>0.6</td>
<td>5</td>
<td>4.3</td>
<td>2.3</td>
<td>3</td>
<td>1.3</td>
<td>1.6</td>
<td>7.3</td>
<td>5.6</td>
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