Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.3, No.5, 2013

Cytogenetic Effects of Benzene on Human Blood Cells

Yasamin Al-Ganimi[1], Ali Al- Saadi[2] , Haider Zaidan[3], Mufeed Ewadh[4*] , Qasim M. Al-Ameri[5]
[1]Dept. of Biology - Karbala university, [2,3] Faculty of Science – Babylon University, , [4]College of Medicine
Babylon University Faculty of Pharmacy – Babylon University
[*]E-mail:mewadh@yahoo.com

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 *etal*.,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 *eta1*., l 998). 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,*etal*.,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,*etal*.,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,*etal*.,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 *etal*.,1994). The soil obtained from oil production facilities and coastal refineries is also highly contaminated by benzene (Eastmond *etal*.,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 *etal*.,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 *etal*.,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 clastiogen. In animal studies, benzene induced cytogenetic effects, including chromosome and chromatid aberrations, sister chromatid exchanges, and micronuclei (Clare *etal*.,1984, Liu *etal*.,2000, Liu *etal*.,2003) There is some evidence that benzene can induce chromosomal abnormalities in mammalian cell cultures (Marcon *etal*.,1999). Metabolites of benzene (hydroquinone, catechol, diolepoxides 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/m^3 (Shuga. *et al*.,2010). However, there was no dose–response relationship in the latter study, as the highest exposure (3.2 mg/m^3) produced fewer aberrations than the middle exposure (32 mg/m^3).

The chromosomal effects in these studies are evident at concentrations of around 320 mg/m^3 (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/m^3 (Sarma, *et al*., 2011). In the study by**.** Martı´nez-Vela´zquez,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/m^3(Martı´nez-Vela´zquez,M. *et al*.,2006). Smoking did not influence the results (Sasiadek .,*et al.,* 1998).

Acknowledgment :We would like to express our great thank to Miss Aalaa A.W.al-bayati and Miss Zeynab Ewadh for Their sincere help in organizing format of this article .

References

Atsdr. (2007) Toxicological Profile for Benzene. http://www.atsdr. cdc.gov/toxprofiles/tp3.html. U.S. Department Of Health And Human Services. Agency for Toxic Substances and Disease Registry, Atlanta, GA. 2.NIOSH. (1990) National Occupational Exposure Survey (1981-83).

Bi,Y. et al. (2009) Gene expression in benzene-exposed workers by microarray analysis of peripheral mononuclear blood cells: induction and silencing of CYP4F3A and regulation of DNA-dependent protein kinase catalytic subunit in DNA double strand break repair. Chem. Biol. Interact, 184, 207–211.

Badham,H.J. *et al*. (2010) In utero and in vitro effects of benzene and its metabolites on erythroid differentiation and the role of reactive oxygen species. Toxicol. Appl. Pharmacol., 244, 273–279.

Baudouin C, Charveron M, Tarroux R, Gall Y(2002): Environmental pollutants and skin cancer. *Cell Biol Toxicol*, 18, 341-348.

Carere A, Antoccia A, Cimini D, Crebelli R, Degrasi F, Leopardi P, Marcon F, Sgura A, Tanzarella C, Zijno A, (1998): Genetic effects of petroleum fuels II: Analysis of chromosome loss and hyperploidy in peripheral lymphocytes of gasoline station attendants. *Environ Mol Mutagen*, 32, 130-138.

Chen H, Rupa DS, Tomar R, Eastmond DA(1994): Chromosomal loss and breakage in mouse bone marrow and spleen cells exposed to benzene *in vivo*. *J Cancer Res*, 54, 3533-3539.

Clare, M.G., A. Yardley-Jones, A.C. Maclean, and B.J. Dean(1984) Chromosome analysis from peripheral blood lymphocytes of workers after an acute exposure to benzene, *Br J Ind Med,* 41, 249–53.
Eastmond DA, Rupa DS, and Hasegawa LS,(1994): Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolour fluorescence *in situ* hybridization with DNA probes. *Mutat Res*, 322, 9-20.

Eastmond DA, Schuler M, Franz Ch, Chen H, Parks R, Wang L, Hasegawa L (2001): Characterization and mechanisms of chromosomal alterations induced by benzene in mice and humans. *Res Rep Health Eff Inst*, 103, 1-68; discussion 69-80.

Gillis,B. *et al.* (2007) Identification of human cell responses to benzene and benzene metabolites. Genomics, 90, 324–333.

Ji,Z. *et al*. (2010) A comparison of the cytogenetic alterations and global DNA hypomethylation induced by the benzene metabolite, hydroquinone, with those induced by melphalan and etoposide. Leukemia, 24, 986–991

Khalade,A. *et al*. (2010) Exposure to benzene at work and the risk of leukemia: a systematic review and meta-analysis. Environ. Health, 9, 31

Liu S, Zheng L, Deng L, and Tang G, Zhong O(2000): Detection of numerical chromosome aberrations in sperm of workers exposed to benzene series by two-color fluorescence *in situ* hybridizaion. *Zhonghua Yu Fang Yi Xue Za Zhi*, 34, 17-19.

Liu XX, Tang GH, Yuan YX, Deng LX, Zhang Q, Zheng LK(2003): Detection of the frequencies in numerical and structural chromosome aberrations in sperm of benzene series-exposed workers by multi-color fluorescence *in situ* hybridisation. *Yi Chuan Xue Bao*, 30, 1177-1182.

Marcon F, Zijno A, Crebelli R, Carere A, Veidebaum T, Peltonen K, Parks R, Schuler M, Eastmond D (1999): Chromosome damage and aneuploidy detected by interphase multicolour FISH in benzene exposed shale oil workers. *Mutat Res*, 445, 155-166

Martı´nez-Vela´zquez,M. *et al*. (2006) Benzene metabolites induce apoptosis in lymphocytes. Exp. Toxicol. Pathol., 58, 65–70.

North,M. *et al*. (2009) Utilizing functional genomics in yeast to discover novel biomarkers of benzene toxicity in humans. Toxicol. Lett., 189Abstracts of the 46th Congress of the European Societies of Toxicology, S93.
Rappaport,S.M. *et al*. (2009) Evidence that humans metabolize benzene via two pathways. Environ. Health Perspect, 117, 946–952

Shuga,J. *et al*. (2010) Selected technologies for measuring acquired genetic damage in humans. Environ. Mol. Mutagen., 51, 851–870.

Sarma,S.N. *et al*. (2011) Differential gene expression profiles of human leukemia cell lines exposed to benzene and its metabolites. Environ. Toxicol. Pharmacol., 32, 285–295.

Sasiadek M, Schlade K, Busza H, Czemarmazovicz H, Stembalska A. (1998). Classical and molecular cytogenetic analysis of diepoxybutaneinduced chromosome aberrations. *Mutat Res.,* 419, 155-161.

Weisel,C.P. (2010) Benzene exposure: an overview of monitoring methods and their findings. Chem. Biol. Interact., 184, 58–66..

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

Zhang,L. *et al*. (2010) Systems biology of human benzene exposure. Chem. Biol. Interact., 184, 86–93.
.

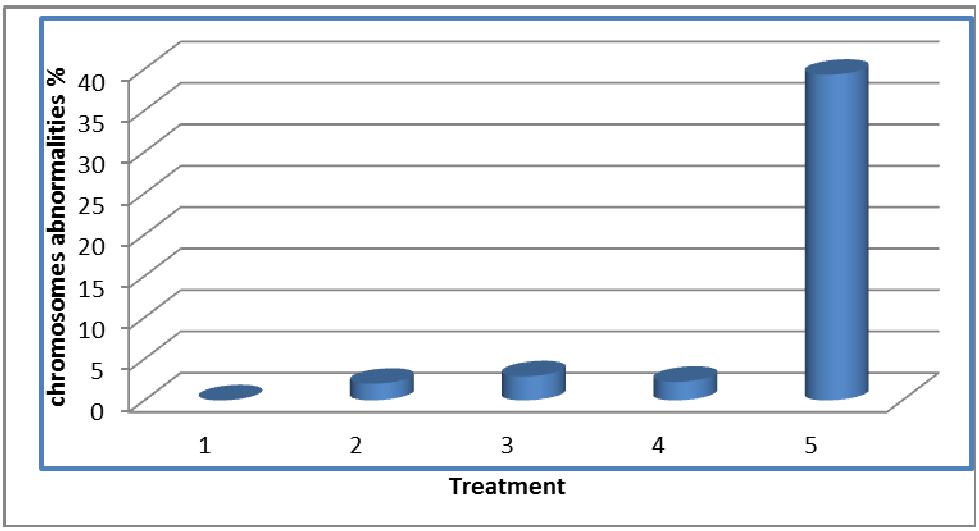

Figure (1): Percentage of chromosomes abnormalities in human blood cells that treated by different concentration of benzene. (1) NC, (2) 2.5×10^{-5}, (3) 5×10^{-5}, (4) 10×10^{-5}, (5) 20×10^{-5}

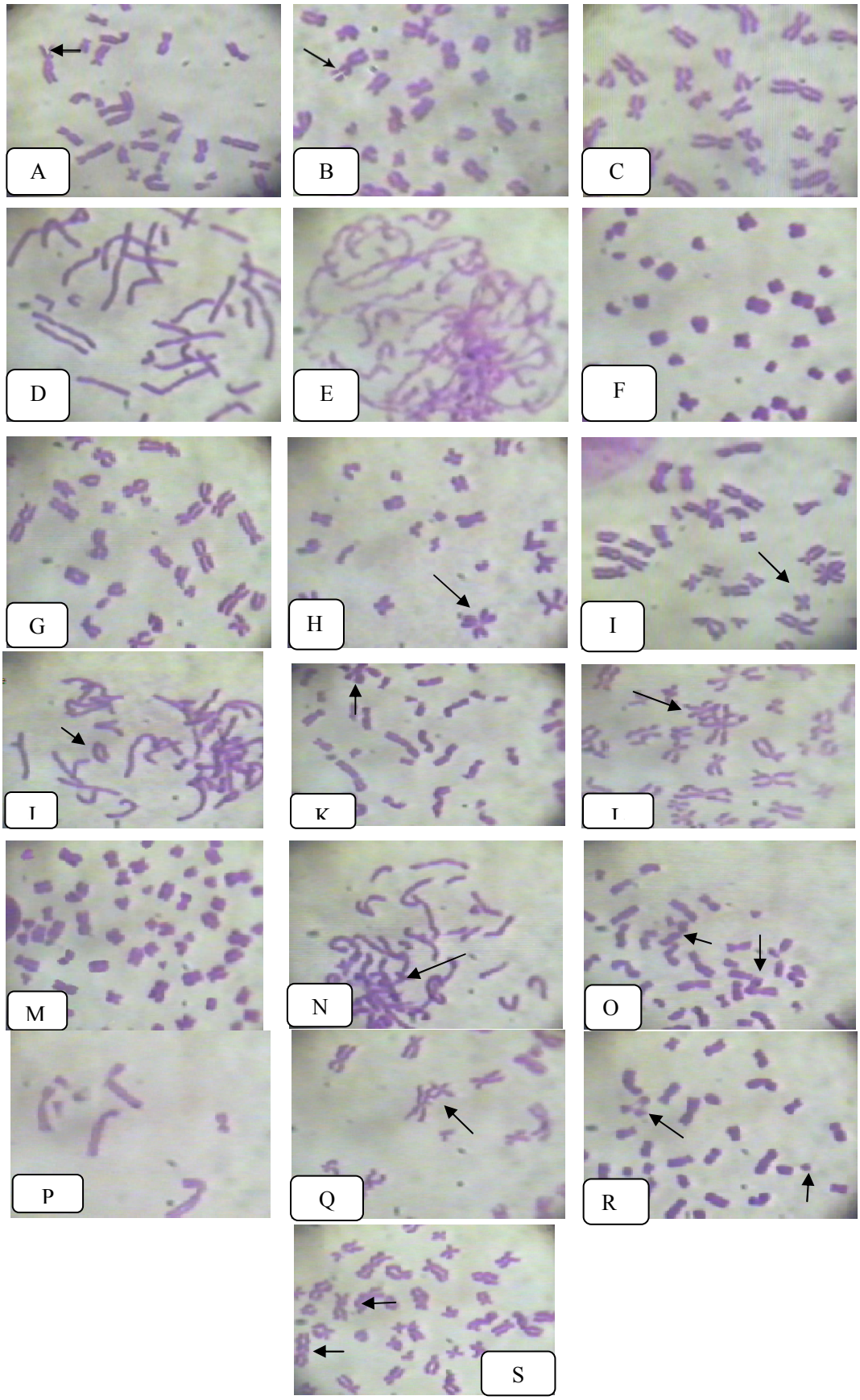

Figure (2) chromosomes abnormalities in human blood cells treated by (10× 10-5 and 20× 10-5 molar) of benzene.

(A) Chromatid break. (B) Chromosomal break. (C) Chromatid deletion. (D,E,F) Bizarre configuration. (G,J) Ring chromosome. (H,I) Centromeres stick. (K,L,N) Chromosome stickiness. (M) Hyperploidy. (O,Q) End association. (P) Aneuploidy. (R) Minute chromosome and (S) Polycentric chromosomes.

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

Treatment	MI
Negative control	8.64
2.5 × 10-5	10.6
5× 10-5	10.6
10× 10-5	10.6
20× 10-5	13.45

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

Treatment	Polycentric chromo	Minute Chromo.	Chromo. stickiness	Ring Chromo.	End to end association	Deletion of chromatid	Bizarre configuration	Deletion	Centromeric association	aneuploidy	Hyperdiploidy	chromatid break	Chrom. break
N. control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.5×10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0	0
5×10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0.6	1.3
10×10^{-5}	0.1	0.1	4	2	0.5	3.7	5.3	4	2.5	1	0.6	3.2	2
20×10^{-5}	0.1	0.2	2.3	3	0.6	5	4.3	2.3	3	1.3	1.6	7.3	5.6

This academic article was published by The International Institute for Science, Technology and Education (IISTE). The IISTE is a pioneer in the Open Access Publishing service based in the U.S. and Europe. The aim of the institute is Accelerating Global Knowledge Sharing.

More information about the publisher can be found in the IISTE's homepage: http://www.iiste.org

CALL FOR PAPERS

The IISTE is currently hosting more than 30 peer-reviewed academic journals and collaborating with academic institutions around the world. There's no deadline for submission. **Prospective authors of IISTE journals can find the submission instruction on the following page:** http://www.iiste.org/Journals/

The IISTE editorial team promises to the review and publish all the qualified submissions in a **fast** manner. All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Printed version of the journals is also available upon request of readers and authors.

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar