Benzene-induced hematotoxicity and DNA damage in rats

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Abstract: Exposure to benzene has a detrimental effect on the hematopoietic system and affects its genetic material through the enhancement of oxidative DNA damage. 8-hydroxy-2-deoxyguanosine (8-OHdG) is a result of DNA damage due to the hydroxyl radical attack at the C8 position of the nucleobase guanine which occurs after exposure to benzene. Such damage if unrepaired, may contribute to mutageninicity and cancer promotion. A group of 19 male Wistar rats were benzene administered by gastric gavage 100 mg / kg body wt, 5d/week for 4 weeks and another group of 12 rats were not exposed to benzene served as control group with the aim to assess the changes in peripheral blood cells count and 8-OHdG levels in benzene exposed rats. There was a highly significant decrease of TLC, RBC and Hemoglobin concentration in benzene exposed group (P=.001,.007,.000), respectively. Also, there was a significant increase (P=.031) of 8-OHdG in benzene exposed group. The mean + (SD) of 8-OHdG in benzene exposed group was (49.5 + 7.1) pg/ml while in control group was (38.7 + 4.3) pg/ml. In conclusion; benzene exposure has a toxic effect on peripheral blood cells with increased the oxidative DNA damage in the form of increased 8-OHdG. Further studies are needed to clarify the biological significance of 8-OHdG as a clastogenic and carcinogenic risk factor.

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Introduction

Long-term exposure to high levels of benzene in the air may affect several parameters related to the hematological system, including the immune system, causing bone marrow depression expressed as anemia, leucopenia (Lan et al., 2004). And thrombocytopenia, leading to pancytopenia and aplastic anaemia (Yu et al., 2007). The U.S. Environmental Protection Agency (EPA) has classified benzene as a known human carcinogen (EPA. 2011).

Benzene is a natural component of crude oil. It was first isolated by Michael Faraday in 1825, and originally produced from coal tar in 1948. Today benzene is mainly produced by catalytic reforming of alkanes and cycloalkanes or by cracking certain gasoline fractions (Yaris et al., 2004). Benzene is emitted during its production, from coke ovens, fuel stations, from different combustion sources, such as motor engines, wood combustion and tobacco smoke, during the manufacture of plastics, resins, paint, varnish, pesticides, detergents, tires, shoes and from evaporation losses during the handling, distribution and storage of petrol (WHO, 2000).

The hematotoxic effect of benzene occurs through its activation in the liver to phenolic metabolites, these metabolites are transported to the bone marrow which is a major target of benzene toxicity (Yoon et al., 2003). Bone marrow contains several peroxidases; the most prevalent is myeloperoxidase which converts the hepatic formed benzene metabolites phenol to secondary several biologically highly reactive and toxic metabolites (Rivedal, 2005), especially hydroquinone, p-benzoquinone, catechol and muconaldehyde, alone or in combination, are reported to be the most potent metabolites in producing toxicity on the hematopoietic

system (Lagorio et al.,1994).

The mechanisms underlying benzene toxicity are not yet fully understood, they are likely to be complicated by various pathways, including those of metabolism, growth factor regulation, oxidative stress, DNA damage, cell cycle regulation, and programmed cell death (Smith et al., 1996). Benzene metabolites as trans, trans-muconic acid (ttMA) were found to be a strong inhibitor of gap junction intercellular communication that disrupt normal hematopoietic development and induce hematotoxicity (Kimet al., 2004).

Benzene is highly genotoxic (clastogenic) in humans and experimental animals by its oxidative ability. During the metabolism of benzene hydroxyl radicals are generated (Forlenza et al., 2006), leading to oxidative damage to tubulin, histone proteins, topoisomerase II, other DNA associated proteins, and DNA itself; and consequent damage including DNA strand breakage, mitotic recombination, aneuploidy and chromosome translocations (Sajous et al., 2008).

The hydroxyl radicals induce a variety of damage to DNA, including oxidized bases which will then be repaired and eliminated in urine. The hydroxyl radicals may attack at the C-8 position of deoxyguanosine in DNA forming 8-OHdG, which often has been used as a biomarker to assess the extent of repair of reactive oxygen species-induced DNA damage in both the clinical and occupational setting (Williams et al., 2002).

The formation of 8-OHdG in DNA has been shown to cause point mutations, especially G-T and A-C base substitutions, and may also cause strand breaks, the inability to repair these breaks leads to genomic instability, carcinogenesis and cell death (McClendona et al., 2007).

Benzene is not only a genotoxic and carcinogenic, but also various epigenetic phenomena may play a role in benzene- oncogenecity. It was noticed that the low-level benzene exposure is associated in normal subjects with DNA methylation changes that resemble the aberrant epigenetic patterns found in malignant cells (Ishihama et al., 2008).

Double-Strand breakage may also result from modification or damage to key proteins associated with DNA topology (Sul et al., 2005), astopoisomerases (Pilger et al., 2006). Phosphorylated histone is one of these modified proteins produced by exposure to benzene and its metabolites, which are associated with genomic instability leading to leukemia (Akagi et al., 2003). We therefore investigated in an animal model the hemotoxic and gentotoxic effect of benzene.

Materials and methods

Chemicals

Benzine (Sigma Aldrich, St. Louis, USA) was administered by gastric gavage in a dose 100 mg/kg/d, 5d/week for 4 weeks (Sigma Aldrich, St. Louis, USA). Cayman's 8-hydroxy-2-deoxy Guanosine (8-OHdG) assay kit was purchased from Cayman's Chemical Co. (USA). All the reagents used in this study were of analytical grade.

Animals

Male Wistar rats with 200–250 g body weight from the animal house of King Saud University, Riyadh, Saudi Arabia were used in this study. Animals were housed in groups in standard clear polycarbonate cages with food and water available *ad libitum*. Animals were kept on a 12-h light-dark schedule (6:00 am–6:00 pm), and all experimental testing was conducted during the light phase from 9:00 am to 12:00 pm. All experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The experimental protocol was approved by the Institutional Animal Use and Care Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Experimental protocol

A total of 31 rats were employed in the study. The animals were divided into two groups; A group of 17 rats were administered 100 mg benzene (Sigma Aldrich, > 99.8% purity) / kg body wt, 5d/week for 4 weeks. Benzene was given in 1 ml of corn oil via gastric gavage. Twelve normal rats without any chemical additives served as control group. Blood was obtained via cardiac puncture following anesthesia with light ether. About two ml of blood was collected and divided into two separate EDTA containing sterile tubes, one for 8-OHdG detection in plasma and the other for complete blood count.

Blood picture

Hematological parameters were studied using an automated Blood Cell Counter (COULTER® LH 500 Hematology Analyzer - Beckman Coulter, USA).

Determination of 8-OHdG levels

8-OHdG is produced by the oxidative damage of DNA by reactive oxygen and nitrogen species and serves as an established marker of oxidative stress. Cayman's 8-hydroxy-2-deoxy guanosine assay kit purchased from Cayman's Chemical Co. (USA) was used. It is a competitive assay that can be used for the quantification of 8-OHdG in serum and tissue homogenate. It recognizes both free 8-OHdG and DNA-incorporated 8-OHdG. This assay depends on the competition between 8-OHdG and 8-OHdG-acetylcholinesterase (AChE) conjugate (8-OHdGTracer) for a limited amount of 8-OHdG monoclonal antibody. All procedures were carried out in accordance with the manufacturer's instructions.

Statistical analysis

The statistical results were computed using the "SPSS" program for statistics.

Results

Benzene administered rat group (15/17) - as two rats were died with nasal and mouth hemorrhage- showed a significant increase (P=.031) of 8-OHdG in benzene exposed group. The mean ± SD of 8-OHdG in benzene exposed group was (49.5 ± 7.1) pg/ml while in control group was (38.7 ± 4.3) pg/ml. Also, there was a highly significant decrease of TLC, RBC and Hemoglobin concentration in benzene exposed group (P=.001, .007, .000), respectively (table 1).

Table 1. 8-OHdG and blood parameters in control and benzene exposed groups and their significance.

	Mean ± SD		P
	Control	Ben. exposed	
OHdG	38.7 + 4.3	49.5 + 7.1	.031*
WBC	19.2+ 2.5	5.3 +.64	.001**
RBC	7.5+.8	6.2 +.4	.007**
HB	12.7 + 1.4	11.6+.3	.000**
MCV	50 + 2.3	53.4 + 3.1	.062
MCH	17 +.9	17.8 + 1.1	.531
PLT	532.2 + 126.7	952.8 + 190.3	.085

**. P value is highly significant at 0.01 level.
*. P value is significant at 0.05 level.

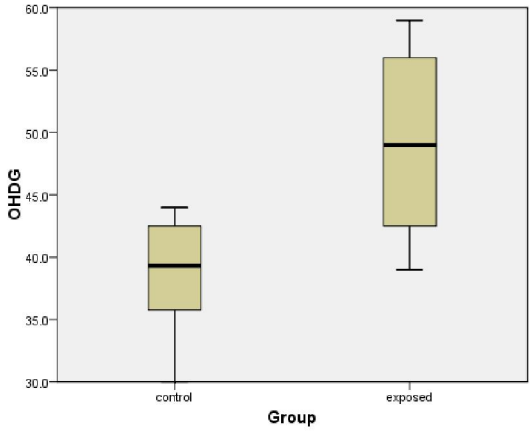

Fig. 1. **Concentrations of 8-OHdG in control and benzene exposed groups**

The mean ± SD of platelet count in benzene exposed group (951.8 ± 190.3) x 10^3 µl is higher than of control group (532.17 ± 126.7) x 10^3 µl. In benzene exposed group there was a positive correlation (r=.639) between 8-OHdG and platelet count while a negative correlation with each of WBC, RBC and hemoglobin concentration (r= -.671, -.508, -.474) respectively (table 2).

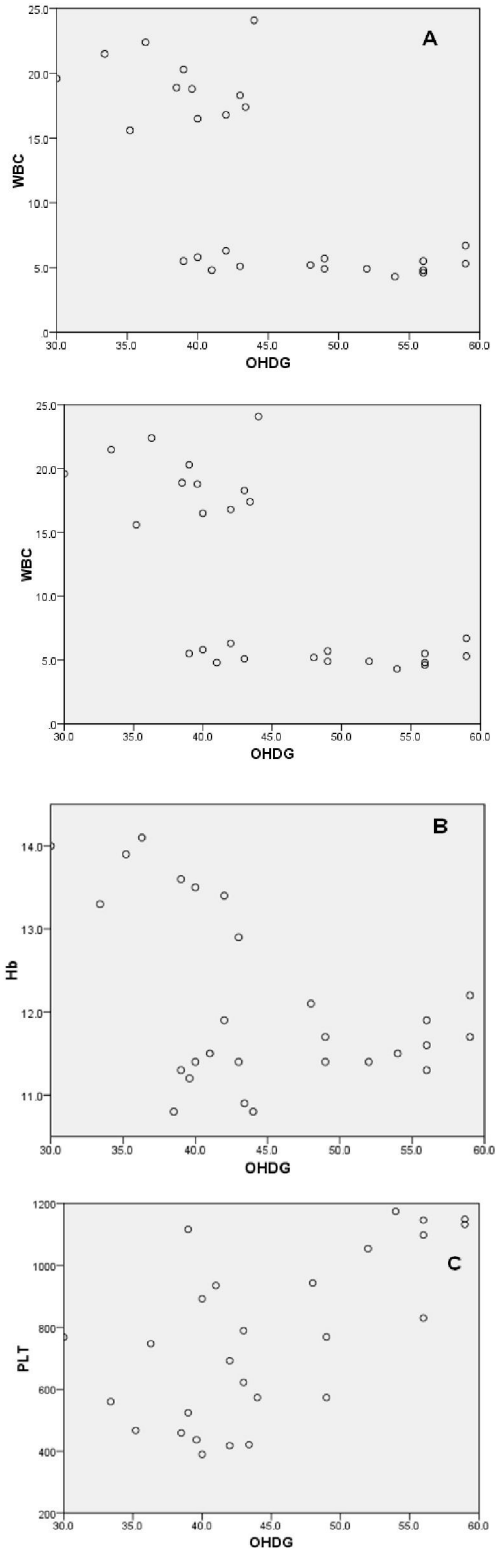

Fig. 2. Shows negative correlation between each of WBCs count and hemoglobin concentration and 8-OHDG levels (A & B), and positive correlation with platelet count (C).

Table 2. The correlation between 8-OHdG and blood parameters.

		TLC	RBC	HB	MCV	MCH	PLT
8-OHdG	r	-.671	-.508	-.474	.192	.311	.639
	P*	.000**	.007**	.013*	.338	.115	.000**

**. P value is highly significant at 0.01 level.
*. P value is significant at 0.05 level.

Discussion

In this study we investigated the hemotoxic and genotoxic effect of benzene. Our results show that in rats, a significant increase of 8-OHdG levels was observed in benzene exposed rats. The mean of 8-OHdG level in benzene exposed group was (49.5 ± 7.1) pg/ml versus (38.7 ± 4.3) pg/ml in control group. Benzene and its metabolites induce an oxidative DNA damage which will then be repaired and eliminated in urine in the form of 8-OHdG (Sperati et al., 1999). 8-OHdG which is the result of hydroxyl radicals attack at the C-8 position of deoxyguanosine has been used as a biomarker to assess the extent of oxidative DNA damage (Hakim et al., 2008).

In this study, 8-OHdG was measured in plasma although many of previous studies measured it in urine. Urinary 8-OHdG levels may be affected by renal impairment (sakano et al., 2009), and need 24 hours urine sample collection and kidney function investigation to exclude any renal disorder (Lin et al., 2004). More over in complex samples such as plasma, 8-OHdG can exist as either free nucleoside or incorporated in DNA.

Once the blood enters the kidney, free 8-OHdG is readily filtered into the urine, while larger DNA fragments remain in the blood stream (Qu et al., 2002), so plasma sample is considered more reflective for oxidative DNA damage than urine sample.

This study showed a highly significant decrease of TLC, RBC and hemoglobin concentration in benzene exposed rats. Previous study reported that the reductions in the various exposure groups compared with the controls were 14.5–26.4% for white blood cells. Also, another study reported a 4.3–29.1 % reduction for white blood cells and13.0–15.6% for red blood cells (Tasi et al., 2004).

Interestingly, several studies also reported no decrease in blood cell counts among benzene-exposed workers or that some of the hematological parameters previously reported to be sensitive to benzene exposure, such as white and red blood cells were in fact significantly increased in the exposed group compared with controls (Ray et al., 2007).

There is a remarkable variation in the mean platelets count between control (532.2 + 126.7) and benzene exposed group (952.8 ± 190.3) with increased mean platelets count in last group.

In benzene exposed group; there was a positive correlation (r=.639) between 8-OHdG and platelet count while a negative correlation with each of WBC, RBC and hemoglobin concentration (r = -.671, -.508, -.474) respectively (table 2). The above results increased the significance of 8-OHdG as a sensitive biomarker for benzene toxic effects on mature blood cells.

It has been posited that benzene and its metabolites mediate the multiple biological effects of benzene on the hematopoietic cells to give rise to bone marrow toxicity. Several studies attributed the myelotoxicity of benzene to some modified proteins "as histones", which were detected particularly in bone marrow of mice following treatment with benzene (Zhao et al., 2009).

DNA adduct formation from benzene metabolites can potentially damage bone marrow cells. On the other hand, the chronic and progressive nature of benzene hematotoxicity suggests genomic reprogramming that would result in aberrant gene expression inducing extensive apoptosis in marrow cells (Zhao et al., 2009). One limitation of our study is that our experiments have thus far been conducted only on rats.

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