

BENZENE INDUCED HEMATOTOXICITY AND RECENT SCENARIOS

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ABSTRACT

Benzene is highly volatile solvent which is found in industries as well as natural sources. Benzene is primarily metabolized in the liver. It is found in a series of phenolic and ring opened products. The mechanism of benzene induced aplastic anemia seems to include the concerted action of different types of metabolites being together on early stem cells and progenitor cells in bone marrow. Benzene is highly clastogenic and carcinogenic solvent. It shows chromosomal aberration, chromatid change and micronuclei. Metabolites of benzene, hydroquinone to benzoquinones in the bone marrow are responsible for the benzene carcinogenicity. It also acts as a slow CNS depressant. The intoxication of benzene is by inhalation, ingestion or cutaneous absorption. Toxic symptoms include cyanosis, methemoglobinemia, vertigo, headache and convulsions.

INTRODUCTION

Benzene is a colorless, highly volatile chemical compound which is found in air, water and soil. We can get it from both industrial and natural sources. Benzene occurs naturally in crude petroleum and it also occurs in processing products like motor fuels and solvents. It is also used in cooking of coal, production of toluene, xylene and other aromatic compounds. The most significant exposure of benzene is through the inhalation process, tobacco and smoking. Benzene has been associated with an acute and long term adverse effects and diseases, including aplastic anemia, leukemia, thrombocytopenia, pancytopenia and cancer. All have been reported after chronic exposure to benzene. Benzene, chemical formula C_6H_6 , has a molecular weight: 78.11 g/mol. Benzene is a chemical that is a colorless or light yellow liquid at room temperature. It has a sweet odor and is highly flammable. It is as yellow to pale brown crystal or as a yellowish oily liquid, with a melting point of 5.5 °C and boiling point of 80.1 °C. Benzene is absorbed from the gastro intestinal tract, through skin and the lungs. Lesser exposures of benzene can be exhaled through lungs, small amount can be

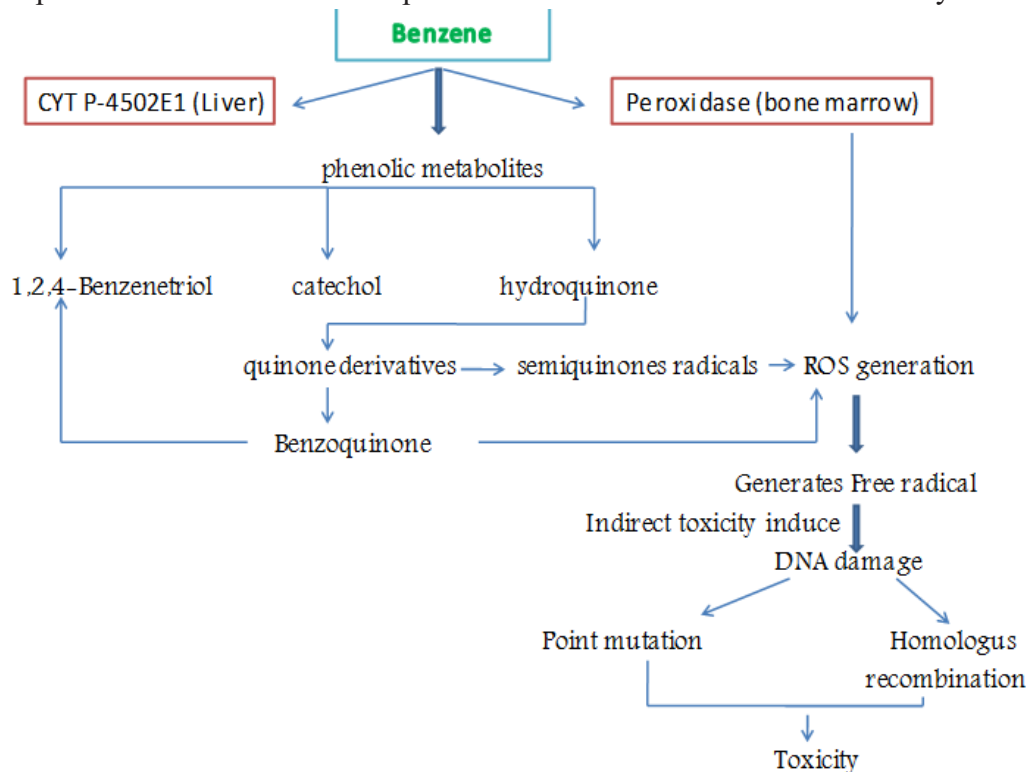
excreted unchanged in urine mainly as phenol, catechol and quinol in conjugated forms.

EFFECT OF BENZENE ON LIVER AND BONE MARROW CELLULARITY

Benzene has toxic metabolites. It is genotoxic and carcinogenic and ubiquitous environmental pollutant (DOAA.). In animal models, liver is the primary target of benzene toxicity ⁽¹⁾ and its secondary metabolites occur in the bone marrow. ⁽²⁻⁶⁾ The free radicals in benzene toxicity have relatively high levels of myeloperoxidase, which are responsible for localization of benzene toxicity in bone marrow. The initial step involving benzene is hydroxylated to phenol, catechol, hydroquinone and 1,2,4-benzenetriol. The products are excreted as ethereal sulfates and glucuronides through urine ⁽⁷⁾. These hydroxylated products of phenol, catechol, and hydroquinones are excellent substrate of myeloperoxidase, since there is high concentration of myeloperoxidase present in the bone marrow. The hydroxylated products of phenol, catechol and hydroxyquinone act as excellent substrate of myeloperoxidase, because of myeloperoxidase is present in very high

concentration in the bone marrow.^(8,9) Metabolism of benzene takes place in the liver, where metabolism is mediated by cytochrom P-450 monooxygenase which results in formation benzeneoxide. The cytochrome p-450 catalyses the benzene metabolites in the formation of benzene oxide^[3-8]. These metabolites which are mediated by cytochrome p-450 are present in limited extent in tissue because of low level of cytochrome p-450 in bone marrow as compared to the liver.^[10] Muconaldehydes may be the metabolites which are formed in the liver⁽¹¹⁾. Metabolism of oxepin is thought to open the aromatic ring and produce muconaldehyde which causes hematotoxicity.^[12] These reactive quinones metabolites and muconaldehydes are electrophiles that directly react with the proteins and cellular nucleophiles

including DNA.^[12,13] Phenolic metabolites of quinones and semiquinones are produced through peroxidases in the bone marrow where these free radicals are very toxic and directly bound to the cellular macromolecules and produce oxygen radicals by redox reaction.^[14,15] Metabolites of benzene, hydroquinone to benzoquinones in the bone marrow are responsible for the benzene carcinogenicity.^[14] Phenol and catechol have been shown to activate myeloperoxidase dependent conversion of hydroquinone to benzoquinones.^[15-17] These phenolic compounds in the liver are transported and accumulated in the bone marrow.^[14] In support of this, evidence shows that the benzoquinones detoxifying enzyme NAD (P) H: quinines oxidoreductase 1 (NQO1) protects benzene induced hematotoxicity.^[17,18]



EFFECTS OF BENZENE ON HEMATOPOIETIC STEM CELLS

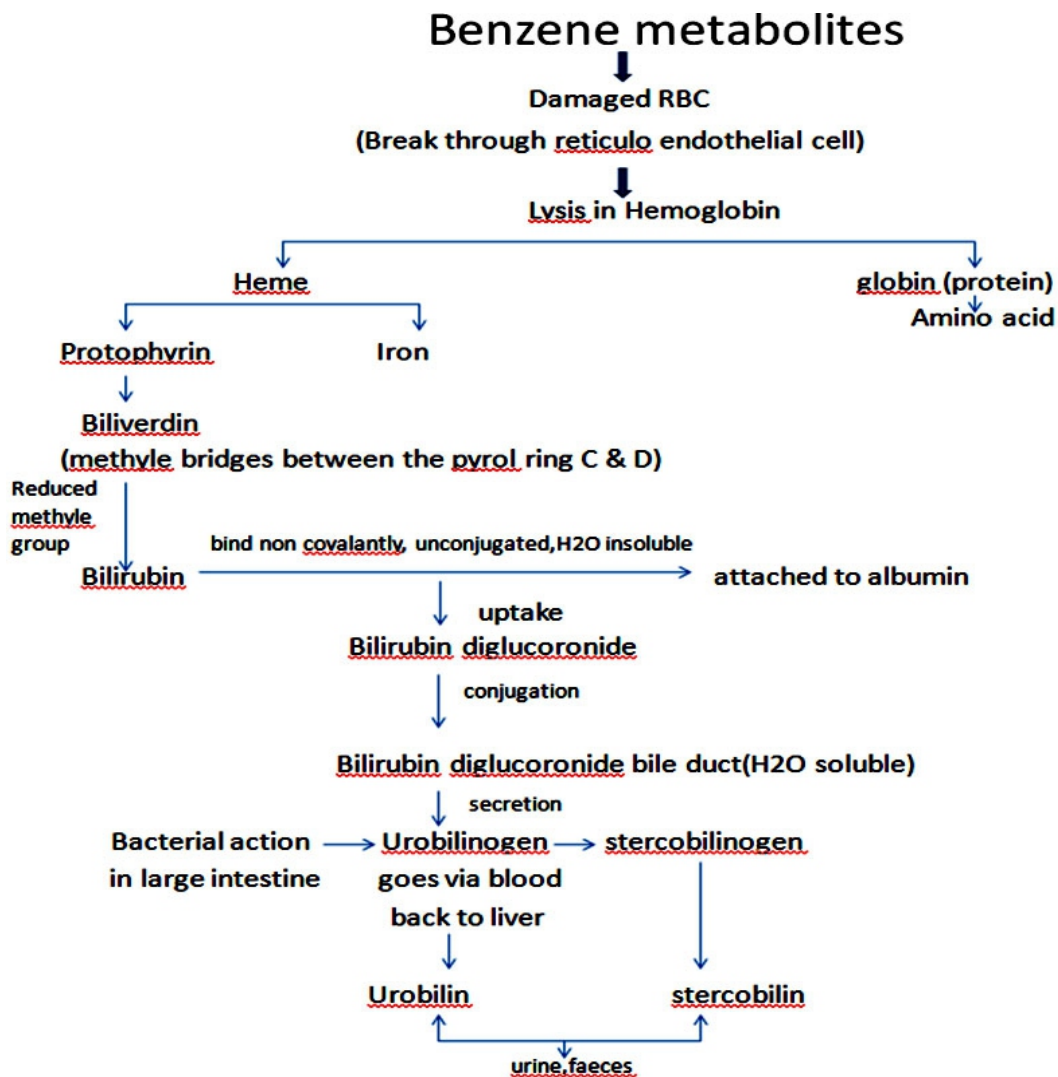
The toxic effects of benzene in animals model used for the study of benzene toxicity has been led to the bone marrow depression causing aplastic anemia. It may be inherited or acquired type of anemia resulting from intravascular and extravascular blood cell destruction. Aplastic anemia is a serious, often fatal, disorder in which the elements of bone marrow are replaced through the fat and reduced blood cells element. Hematopoietic stem cells which are present in the bone marrow produce major cell types i.e. erythrocytes, thrombocytes and

leukocytes.^[18] Maturation and activation of blood cells occur in secondary lymphoid organ (spleen, thymus and lymph node). Decrease in white blood cells is known as leucopenia and decrease in platelets count is called thrombocytopenia. Red blood cells of hemoglobin transport the oxygen from lung to tissues. The significant decrease in the blood cells causes death due to hemorrhage and decrease in red blood cells count (anemia). Bone marrow damage due to the toxicity of benzene metabolites reduces the oxygen carrying capacity of blood resulting in the decrease in red blood cells, iron deficiency anemia. Reduction in the volume

packed red cells is calculated by dividing the hematocrit through the red blood cells count. Increase in the packed cell volume is observed in almost any form of aplastic anemia. Due to the deficiency of folic acid and vitamin B₁₂ it causes packed cell volume to increase. Benzene metabolites, which are produced by various metabolism in the liver and bone marrow, damage hematopoietic stem cells. Oxyhemoglobin can be oxidized to methemoglobin through benzene-induced processes. Methemoglobin is unable to bind to oxygen. Methemoglobin is converted into deoxyhemoglobin in anaerobic systems or the formation of oxyhemoglobin occurs in aerobic system. The oxidation of benzene metabolites leads to bring reactive oxygen species (ROS) and a complex array of benzene derived free radicals.^[20] Not only ROS, benzene metabolites causing lipid peroxidation and protein oxidation when react with plasma membrane molecules result in the destruction of RBCs and hemolytic anemia.^[17-19]

MECHANISM OF HEMOLYSIS

The physiological process of RBC (shown in fig. 2) gives better understanding of hemolysis : breakdown in RBC through the toxic metabolites of benzene, damaged RBC, phagocytosed through the reticulo endothelial cells where as hemoglobin is lysed into heme and globin. Then heme is converted into iron. Iron is transferred through reticulo-endothelial cell into blood. In bone marrow, iron is used to make new red blood cells (erythrocyte). Ironless part of heme is changed into biliverdin (green in color) after then this biliverdin is changed into bilirubin with some carbon monoxide which is expired through the lungs. Bilirubin is circulated into liver, is excreted into the gut via the bile. Bacteria present in large intestine changed the bilirubin into urobilin (yellow in color) which is excreted by urine. The remaining part of urobilin is converted into stercobilin (brown in color) which is excreted in the faeces.



CONCLUSIONS AND PROSPECTS

The treatment of severe aplastic anemia, whether by allogeneic stem-cell transplantation or immunosuppression has improved dramatically over the last 25 years, and long-term survival of more than 75% of patients can be anticipated with either therapy. The number and types of blood cells were examined in seven day study in which peripheral lymphocytes, RBC, WBC, PLT and Hb level were reduced. There were various changes in hematological parameters under investigation after the administration of benzene in comparison to the control groups. Benzene can be measured in those people blood who work in industries. Benzene rapidly disappears in the blood, measurements may be useful only for recent exposures. In our body, benzene is converted to products called metabolites. Benzene metabolites like phenol, muconic acid and S-phenylmercapturic acid can be measured in the urine. The amount of phenol in urine has been used to check for benzene exposure in workers. The measurement of benzene in blood or of metabolites in urine cannot be used for making predictions about whether you will experience any harmful health effects. Blood counts of all components of the blood and examination of bone marrow are used to determine benzene exposure and its health effects. For people exposed to relatively high levels of benzene, complete blood analyses can be used to monitor possible changes related to exposure. However, blood analyses are not useful when exposure levels are low. For the analysis of the lower exposures of the chemical such as benzene and its derivatives like aniline, phenol etc. the urine, vitreous humor and bile are the major sources. The presence of the benzene in the body fluids can be analyzed by using various techniques starting from color test, UV spectrometer and IR spectrometer etc.

The benzene has been known to have toxicity on blood. There are various substances which are useful in getting all the parameters back. The products used for getting the hematological parameters back can be either natural, semi synthetic and synthetic. They may be the plant products like *Plumbago* sp, *Eclipta* sp, *Asparagus* sp, vitamin C, iron supplements etc.

The administration of Vitamin C is capable of suppressing benzene induced hematotoxicity in rats. The beneficial effects of vitamin C may be

mediated by its antioxidant effect. Antioxidant treatment with vitamin C significantly reduced the lipid peroxidation as well as increased the superoxide dismutase and catalase activities in rat brain. Our findings suggest that supplementation of ascorbic acid reverse the hematotoxicity and oxidative stress induced by carbamazepine and also imply that a strong protective effect could be achieved using vitamin C. The foregoing indicates that, as an antioxidant agent, vitamin C may have inhibited the chain reactions of chemical agent-generated free radicals or scavenged the reactive free radicals before they reached their hepatic targets by preventing ROS damage in polyunsaturated fatty acids as a liposoluble antioxidant and acting against damage caused to phospholipids as a membrane-stabilizing agent. In addition, vitamin C is known to act by breaking the antioxidant chain that prevents ROS-produced cell membrane damage. The ameliorating effects of vitamins C on benzene-induced hepatotoxicity and hematotoxicity are likely to be mediated via the inhibition of free radical generation and free radical scavenging activity.

REFERENCES

1. Debra L. Laskin, Diane E. Heck, Chitra J. Punjabi, and Jeffrey D. Laskin: Role of Nitric Oxide in Hematosuppression and Benzene-induced Toxicity, *Environmental Health Perspectives* - 104, (6 December 1996)
2. Sammett D, Lee EW, Kocsis JJ, Snyder R. Partial hepatectomy reduces both metabolism and toxicity of benzene. *J Toxicol Environ Health*. 5(5):785–792 (1979 Sep).
3. Andrews LS, Lee EW, Witmer CM, Kocsis JJ, Snyder R. Effects of toluene on the metabolism, disposition and hemopoietic toxicity of [3H]benzene. *Biochem Pharmacol*. 26(4):293–300, (1977 Feb 15).
4. Irons RD, Dent JG, Baker TS, Rickert DE. Benzene is metabolized and covalently bound in bone marrow in situ. *Chem Biol Interact*. 1980 May; 30(2):241–245. Subrahmanyam
5. V.V., Ross, D., Eastmond., D. and Smith, M.T. Hydroxylation of Phenol to Hydroquinone Catalyzed by A Human Myeloperoxidase-Superoxide Complex: Possible Implications in Benzene-Induced Myelotoxicity *Free Radical Research Communications*, 15:285-296 (1991).

6. Jaffe, M. Uber die Aufspaltung des Benzolrings im Organismus. 1. Mit- teilung. Das Auftreten von Muconsaure im Harn nach Darreichung von Benzol. Hoppe-Seyl. Z. Physiol. Chem. 62: 58-67 (1909).
7. DA Eastmond, Smith, M.T., Ruzo, L.O., and Ross, D. Metabolic activation of N-hydroxy arylamines and N-hydroxy heterocyclic amines by humansulfotransferase (s) Pharmacol. 91:85-95(1986)
8. Eastmond D. A., Smith, M. T., and Irons, R. D. An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene exposure. Toxicol. Appl. Pharmacol. 91: 85-95 (1987).
9. Mueller, G., Koelbel, M., Heger, M., and Norpoth, K. Urinary S- phenylmercapturic acid and phenylguanine as indicators of benzene exposure. In: Biological Monitoring of Exposure to Chemicals. Organic Com- pounds (M. H. Ho and H. K. Dillon, Eds.), John Wiley and Sons, New York, pp. 91-98, (1987).
10. Irons, R., Dent, J., Baker, T., and Richert, D. Benzene is metabolized and covalently bound in bone marrow in situ. Chem.-Biol. Interact. 30: 241-245 (1980).
11. Latriano, L., Goldstein, B. D., and Witz, G. Formation of muconaldehyde, an open-ring metabolite of benzene in mouse liver microsomes: a novel pathway for toxic metabolites. Proc. Natl. Acad. Sci. U.S.A. 83: 8356-8360 (1986).
12. G. Witz (1996) Reactive ring-opened aldehyde metabolites in benzene hematotoxicity. Environmental health perspectives, vol. 104, (suppl.6), 1195-1199
13. Jowa, L., Witz, G., Snyder, R., Winkle, S., and Kalf, G. F. Synthesis and characterization of deoxyguanosine-benzoquinone adducts. J. Appl. Toxicol. 10: 47-54 (1990).
14. Dorshkind K. Regulation of hemopoiesis by bone marrow stromal cells and their products. Annu. Rev. Immunol. 8: 111-137(1990). McHale CM
15. Zhang L, Smith MT. Current understanding of the mechanism of benzene-induced leukemia in humans: implications for risk assessment. Carcinogenesis; 33(2):240-52(2012).
16. Subrahmanyam VV, Ross D, Eastmond D, Smith MT. Potential role of free radicals in benzene-induced myelotoxicity and leukemia. Free Rad Biol Med, 11: 495 -515 (1991).
17. Bonnet, D.; Dick, J.E. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. 3, 730-737 (1997).
18. Ahmed Deldar and Charles E. Stevens Development and Application of In Vitro Models of Hematopoiesis to Drug Development Toxicology pathology, 21(1993).
19. Debra L. Laskin, Diane E. Heck, Chitra J. Punjabi, and Jeffrey. Role of Nitric Oxide in Hematosuppression and Benzene-induced Toxicity (Environ Health Perspect 104(Suppl 6):1283-1287 (1996).
20. Martyn T. Smith. The Mechanism of Benzene-induced Leukemia: A Hypothesis and Speculations on the Causes of Leukemia Environ Health Perspect 104(Suppl 6):1219-1225 (1996).