Chemico-Biological Interactions xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

BENZENE 2009—Health effects and mechanisms of bone marrow toxicity: Implications for t-AML and the mode of action framework

Michael G. Bird[a], Helmut Greim[b], Debra A. Kaden[c], Jerry M. Rice[d,*], Robert Snyder[e]

[a] ExxonMobil Biomedical Sciences, Annandale, NJ, USA
[b] Technical University of Munich, Freising-Weihenstephan, Germany
[c] DakTox LLC, Arlington, MA, USA
[d] Georgetown University Medical Center, Washington, DC, USA
[e] Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ, USA

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Benzene
Bone marrow
Aplastic anemia
Acute myeloid leukemia (AML)
Toxicology
Epidemiology
Mode of action
Therapy-related AML (t-AML)

ABSTRACT

This overview of the Symposium and its organization includes a historical survey of the scientific literature in which the relationship of benzene exposure to the development of aplastic anemia and other bone marrow diseases, including acute myeloid (myelogenous) leukemia, is described. Previous conferences on the health effects of benzene are summarized. The important role of the revised World Health Organization classification of tumors of the hematopoietic and lymphoid tissues in clarifying the specific diseases related to benzene exposure is emphasized.

1. Introduction: benzene as a bone marrow toxicant

It has been a little more than a century since the effects of chronic industrial exposure to benzene were first reported by Santesson [1] who described the fate of four women who worked in a tire factory where benzene was used as a solvent. They manifested severe defects in blood clotting, which he described as purpura haemorrhagica. Similar reports were published in the same year by Lenoir and Claude [2] and later by Selling [3]. Today we would recognize their condition as aplastic anemia. Indeed, Santesson described benzene as ". . .das wesentliche toxische Prinzip. . .", i.e., the intrinsic toxic principle, responsible for producing their disease. The first animal models which permitted the study of the bone marrow depressant effects of benzene were developed by Selling [4] using the subcutaneous injection route in rabbits, and by Weiskotten [5] who administered benzene via inhalation. In the early years of the 20th century several reports appeared demonstrating that benzene exposure resulted in impairment of the immune system [6–10].

Through the efforts of Hamilton [11–13], a pioneering figure in occupational medicine, the medical community was made aware of the dangers of chronic exposure to benzene in the workplace. The alarm raised by Hamilton stimulated studies in many workplaces over the next few decades which were descriptive of benzene-induced blood dyscrasias, e.g., Greenburgh et al. [14], Hamilton-Patterson and Browning [15], Helmer [16], Savilahti [17], and Aksoy et al. [18].

2. Benzene as a leukemogen

It took longer for the concept that benzene exposure could also result in leukemia to become accepted. Although there was a report linking benzene exposure to leukemia by Delore and Borgomano [19], benzene-induced leukemogenesis was not the subject of the intense study devoted to benzene-induced bone marrow depression leading to aplastic anemia. While our knowledge of bone marrow function and the technology for studying both normal and aberrant bone marrow improved steadily early in the 20th century, the study of the leukemias was at a more primitive stage.

Thus, despite the periodic appearance of reports attempting to link benzene exposure to leukemogenesis [20–22] it required the concentrated efforts of Professor Vigliani in Italy and Professor Aksoy in Turkey to convince the students of benzene-induced bone marrow damage that benzene was also a bone marrow car-

* Corresponding author at: Georgetown University Medical Center, Washington, DC. Mailing address:3213 Coquelin Terrace, Chevy Chase, MD 20815, USA.
Tel.: +1 301 986 0659.
E-mail addresses: jmricewas@aol.com, jr332@georgetown.edu (J.M. Rice), rsnyder@eohsi.rutgers.edu (R. Snyder).

0009-2797/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2009.12.001

cinogen which could cause one or more types of leukemia as well as cytopenias leading to aplastic anemia.

An early stimulus to investigations of benzene as a leukemogen was the report by Penati and Vigliani [23], at the University of Torino, that distinguished between aplastic anemia, "pseudo-aplastic anemia" and leukemia in benzene-exposed workers. Vigliani's work was interrupted by World War II, but in the post-war period he was appointed as Director of Occupational Health at the University of Milano where he became a strong advocate for the concept that benzene exposure could result in occupational leukemogenesis [24].

Professor Vigliani was given strong support through the work of Professor Aksoy who oversaw a major hematological clinic at the University of Istanbul Medical School. Many of the cases, which had been reported by Vigliani in Italy demonstrating both aplastic anemia and leukemia in populations of workers exposed to benzene occurred in shoe factories where a benzene-based glue was used to cement together the components of shoes. Whereas shoes in Turkey had previously been cemented with petroleum-based glues a change to benzene-based glues occurred in the period of 1955–1960 [25]. Soon the hematology clinic in Istanbul began seeing large numbers of cases of chronic benzene poisoning and it was possible for Aksoy and his coworkers to demonstrate that among many thousands of such workers a significant number of cases of both aplastic anemia and leukemia were detected [18,26,27].

The work of Vigliani, Aksoy and others stimulated the U.S. Occupational Safety and Health Administration (OSHA) to initiate plans to regulate industrial benzene exposure. However, it was not possible to rely solely on the reports of Vigliani and Aksoy to support development of new acceptable occupational exposures. OSHA, therefore, undertook a study of rubber workers in Akron Ohio who had been exposed to benzene between 1940 and 1949 and demonstrated a statistically significant increase in leukemia among these workers [28]. A similar observation was made by Ott et al. [29] among benzene-exposed workers at Dow Chemical Company. The most powerful studies of the association between benzene exposure and leukemogenesis, however, were performed by a team in China led by Drs. S.-N. Yin and G.-L. Li [30,31] in collaboration with colleagues at the U.S. National Cancer Institute [32]. A study involving a total of 100,000 workers demonstrated that benzene exposure resulted in a significant increase in cases of aplastic anemia, myelodysplasia, acute myelogenous leukemia and malignant lymphoma.

3. Previous international symposia on health effects of benzene

By the 1970s and 1980s it had become clear that occupational and environmental exposure to benzene had become a world wide health problem and a forum was needed in which basic medical scientists, clinicians and regulatory officials could meet to discuss new observations and research aimed at understanding all aspects of the toxicology of benzene and its impact on human health. Among the first of these was a Symposium on the Toxicology of Benzene and Alkyl Benzenes [33], sponsored by the Industrial Health Foundation (Pittsburgh, PA) in which some of the first attempts to explore the relationship of benzene metabolism to benzene toxicity appeared. The most influential conference on benzene, however, was reported in the IARC Monographs [34] where international consensus that benzene was a human carcinogen was achieved, a consensus that was updated in October 2009, 1 month after this Symposium.[1]

The following symposia and their published proceedings stimulated the organization of this conference:

A Symposium on Benzene Metabolism, Toxicity and Carcinogenesis, NIEHS, March 14–16, 1988, Research Triangle Park, N.C. Environ. Health Perspect. 82, July 1989.
Benzene'95: An International Conference on Benzene Toxicity, Carcinogenesis, and Epidemiology, June 17–20, 1995, The Environmental and Occupational Health Sciences Institute of Rutgers The State Univ. of New Jersey and the Univ. of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ. Environ. Health Perspect. 104 (Suppl. 6), 1996.
The Benzene State-of-the-Art Workshop, December 16–17, 1998, Univ. of Ottawa, Ottawa, Ontario, Canada. J. Toxicol. Environ. Health 61, Part A, 2000.
The International Symposium on Recent Advances in Benzene Toxicity, October 9–12, 2004, the Technical University of Munich, Munich, Germany. Chem. Biol. Interact. 153–154, 2005.

4. Organization of the international Symposium: BENZENE 2009

Major recent scientific advances, including improved diagnostic criteria for hematopoietic and lymphoid neoplasms, the conclusion of major new epidemiologic studies of these diseases and their relation to benzene exposure, and a significant increase in benzene-related studies in the toxicological literature, signified that in 2009 the time was right for the next international benzene Symposium, 5 years after the previous such meeting.

The Organizing Committee agreed the Symposium should open with a discussion of recent advances in understanding bone marrow function with respect to the regulation of stem cell development, the bone marrow microenvironment and a description of the various tumors of the hematopoietic and lymphoid systems as currently classified by the World Health Organization [35]. The subject of therapy-related leukemia (t-AML), which appears to resemble benzene-induced leukemia and consequently may provide more insight into the mechanism of benzene-induced leukemia, was reviewed. The deliberations continued with presentations on the latency period of benzene-induced leukemia, chromosome alterations in myelodysplastic syndrome (MDS) and t-AML, epigenetic gene regulation and micro-RNAs in MDS and AML.

4.1. Epidemiology

A number of epidemiological studies were offered beginning with an analysis of three nested case control studies in the petroleum industry. Next came a review of the "Shanghai Health Study" which was a just-concluded large-scale hospital-based evaluation of hematological diseases in Shanghai using the WHO classification for diagnosis, coupled with an analysis of risk factors including benzene exposure in a variety of environmental settings. An analysis was presented which explored the potential role of benzene as a causative factor in childhood leukemia. There was also a description of a study of workers in a chemical plant in the Netherlands where extensive data on blood cell levels in workers had been collected, and emphasis placed on exposures in the range of 1 ppm or lower. Analysis of the data failed to reveal leucocytopenia in the workers at these low doses. Benzene exposures in various environmental circumstances and specific exposures in Thailand were

[1] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 100F (October, 2009). The Working Group concluded that there is sufficient evidence of a causal association of benzene exposure with ANLL (acute non-lymphocytic leukemia), and limited evidence for ALL, CLL, MM and NHL (see Baan et al. [36]).

Please cite this article in press as: M.G. Bird, et al., BENZENE 2009—Health effects and mechanisms of bone marrow toxicity: Implications for t-AML and the mode of action framework, Chem. Biol. Interact. (2010), doi:10.1016/j.cbi.2009.12.001

described. A presentation was directed to biomarkers as mechanistic probes and was focused on benzene toxicity.

4.2. Mechanistic studies

Mechanistic studies relating to benzene metabolism included a report on the fate of benzene oxide; a description of human benzene metabolism; modeling of the formation of benzene metabolites; and influence of co-exposure with toluene on benzene metabolism and genotoxicity. A review of the growing significance of glutathione adducts of benzene metabolites and their role in inducing oxidative stress in bone marrow was followed by a discussion of the toxicogenomics of benzene from the perspective of a "systems biology" approach.

Mechanistic emphasis was then placed on genetically altered mice with respect to mode of action of benzene in a variety of genetically defined and modified mice. The role of the Ah receptor in the regulation of hematopoiesis in benzene toxicity was presented as an example of the relationship of benzene exposure to signal transduction. Benzene was also shown to impact on embryonic signaling pathways. These presentations were accompanied by a discussion of metabolic factors in determining susceptibility to benzene.

Mechanistic considerations also included DNA-related issues in benzene exposure. The DNA repair issue was approached from the point of view of benzene exposure and in t-AML, and the role of topoisomerase II inhibition and related DNA damage was the subject of critical analysis. In addition there was a discussion of the impact of benzene on cell–cell communication via an effect on gap junctions. Based on mechanistic considerations it has been suggested that benzene also affects proliferating cells of the reticulo-endothelial system outside the bone marrow and by that possibly induces lymphomas.

A series of papers was presented based on selection from a group of volunteer reports submitted for presentation as posters. These related to additional studies in Shanghai on the development of severe aplastic anemia; modulation of hydroquinone-induced benzene toxicity; methylation of tumor suppressor genes in benzene poisoning; and an estimate of the contribution of benzene to the overall burden of occupational cancer in the United Kingdom.

4.3. Mode of action analysis

The final session was devoted to a mode of action analysis on benzene. It opened with a re-evaluation of the concept of the "vanishing zero", reflecting the concept that as analytical techniques become more sensitive, and lower and lower concentrations of chemicals in our environment can be measured, the concept of zero exposure disappears. A report was presented on the concept of thresholds in the age of genomics where it was shown that it is possible to detect exposures to carcinogens at which no alterations in transcription (NOTEL) and which no adducts on DNA can be detected (NODAL) are observed. The conference terminated with an open panel discussion aimed at applying the IPCS Mode of Action/Human Relevance framework to benzene. Evolving data on benzene were considered in the context of proposed key events in a hypothesized mode of action in animals and their human relevance in the interest of additionally coordinating and focusing research on critical data gaps in a risk assessment context.

5. Summary

The intent of the Organizing Committee was first to offer a forum for presenting the current state of understanding of benzene-induced bone marrow damage. Both laboratory research and studies in human populations were presented. Some of the more challenging areas included observations on the effects of benzene exposure on signal transduction mechanisms in bone marrow, factors leading to susceptibility of humans to benzene-induced diseases, comparisons of benzene-induced leukemias and leukemias observed in humans following anti-cancer chemotherapy, and potential effects of low dose exposure. Discussions were both vigorous, rigorous, and often demonstrated differences in the interpretation of the data. In the final analysis these proceedings can lay the groundwork for further research on the benzene problem over the next decade, as well as on factors leading to leukemogenesis in the general population.

It is clear that while substantial progress has been made in further defining benzene health effects at both the clinical and molecular levels, more mechanistic research is needed to identify the key events in what is evidently a multistep and complex process for benzene-induced leukemia. It is now possible to frame available knowledge in the context of the mode of action/human relevance framework, which is designed to consider weight of evidence of mechanistic data in a risk assessment context. This approach is anticipated to contribute to better coordination of epidemiological and toxicological research and identification of critical data gaps. This process should result in improved quantitative risk estimates for human populations, and will constructively support ongoing regulatory efforts to minimize the adverse health effects of benzene.

References

[1] C.G. Santesson, Über chronische Vergiftungen mit Steinkohlenteerbenzin: vier Todesfälle, Arch. Hyg. Berl. 31 (1897) 336–376.
[2] M.M. Lenoir, H. Claude, A case of purpura attributed to benzene poisoning, Bull. Med. Hôp. Paris 14 (1897) 151–160 (As cited in B.T. Golding, W.P Watson, Possible mechanisms of carcinogenesis after exposure to benzene, in: B. Singer, H. Bartsch (Eds.), Exocyclic DNA Adducts in Mutagenesis and Carcinogenesis, IARC Scientific Publications, IARC, Lyon, 1999).
[3] L. Selling, A preliminary report on some cases of purpura haemorrhagica due to benzol poisoning, Johns Hopkins Hosp. Bull. 21 (1910) 33–37.
[4] L. Selling, Benzol as a leucotoxin, Johns Hopkins Hosp. Rep. 17 (1916) 83–148.
[5] H.G. Weiskotten, T.C. Wyatt, R.F.D. Gibbs, The action of benzol. VI. Benzol vapor leucopenia (rabbit), J. Med. Res. 41 (1920) 425–438.
[6] W.E. Camp, E.A. Baumgartner, Inflammatory reactions in rabbits with severe leucopenia, J. Exp. Med. 22 (1915) 174–193.
[7] L. Hektoen, The effect of benzene on the production of antibodies, J. Infect. Dis. 19 (1916) 69–84.
[8] M.C. Winternitz, A.D. Hirschfelder, Studies upon experimental pneumonia in rabbits, Parts I–III, J. Exp. Med. 18 (1913) 657–665.
[9] J.P. Simonds, H.M. Jones, The effect of injections of benzol upon the production of antibodies, J. Med. Res. 33 (1915) 197–209.
[10] W.C. White, A.M. Gammon, The influence of benzol inhalations on experimental tuberculosis in rabbits, Trans. Assoc. Am. Phys. 29 (1914) 332–337.
[11] A. Hamilton, The growing menace of benzene (benzol) poisoning in American industry, J. Am. Med. Assoc. 78 (1922) 627–630.
[12] A. Hamilton, The lessening menace of benzol poisoning in American industry, J. Ind. Hyg. 10 (1928) 227–233.
[13] A. Hamilton, General review: benzene (benzol) poisoning, Arch. Pathol. 11 (1931) 434–647.
[14] L. Greenburgh, M.R. Mayers, L. Goldwater, A.R. Smith, Benzene (benzol) poisoning in the rotogravure industry in New York City, J. Ind. Hyg. 21 (1939) 385–420.
[15] J.L. Hamilton-Patterson, E. Browning, Toxic effects in women exposed to industrial rubber solutions, Br. J. Ind. Med. 1 (1944) 349–352.
[16] K. Helmer, Accumulated cases of chronic benzene poisoning in the rubber industry, Acad. Med. Scand. 118 (1944) 254–275.
[17] M. Savilahti, More than 100 cases of benzene intoxication in a shoe factory. Observations on hematological symptoms and course of the process in one year, Gewerbehygiene 5 (1956) 147–157.
[18] M. Aksoy, K. Dincol, T. Akgun, S. Erdem, G. Dincol, Hematological effects of chronic benzene, Br. J. Ind. Med. 28 (1971) 296–302.
[19] P. Delore, C. Borgomano, Leucemie aigue au cours de la intoxication benzenique, J. Med. Lyon 9 (1928) 225 (As cited in: A. Hamilton, General review: benzene (benzol) poisoning. Arch. Pathol. 11 (1931) 434–454, 601–637).
[20] E.H. Falconer, An instance of lymphatic leukemia following benzol poisoning, Am. J. Med. Sci. 186 (1933) 353–361.
[21] G.O.E. Lignac, Benzene induced leukemia in humans and white mice, Klin. Wochenschr. 12 (1933) 109–110 (The paper was originally written in German. It was helpful to have available an English translation prepared by Leo Kanner Associates, Redwood City, CA, 1977).
[22] T.B. Mallory, E.A. Gall, W.J. Brickley, Chronic exposure to benzene (benzol). III. The pathologic results, J. Ind. Hyg. Toxicol. 21 (1939) 355–393.

Please cite this article in press as: M.G. Bird, et al., BENZENE 2009—Health effects and mechanisms of bone marrow toxicity: Implications for t-AML and the mode of action framework, Chem. Biol. Interact. (2010), doi:10.1016/j.cbi.2009.12.001

[23] F. Penati, E.C. Vigliani, Sul problema delle mielopatie asplastiche pseudoaplastiche e leucemiche da benzolo, Rass. Med. Ind. 9 (1938) 345–361.
[24] E.C. Vigliani, G. Saita, Benzene and leukemia, N. Eng. J. Med. 271 (1964) 872–876.
[25] M. Aksoy, Benzene hematotoxicity, in: M. Aksoy (Ed.), Benzene Hematotoxicity, CRC Press, 1988, pp. 104–112.
[26] M. Aksoy, K. Dincol, S. Erdem, G. Akgun, G. Dincol, Details of blood changes in 32 patients with pancytopenia associated with long term exposure to benzene, Br. J. Ind. Med. 29 (1972) 56–64.
[27] M. Aksoy, S. Erdem, G. Dincol, Leukemia in shoe-workers occupationally exposed to benzene, Blood 44 (1974) 837–841.
[28] P.F. Infante, R.A. Rinsky, J.K. Wagoner, R.J. Young, Leukemia in benzene workers, Lancet 2 (1977) 76–78.
[29] M.G. Ott, J.C. Townsend, W.A. Fishbeck, R.A. Langner, Mortality among individuals occupationally exposed to benzene, Arch. Environ. Health 33 (1978) 3–10.
[30] S.-N. Yin, Q. Li, Y. Liu, F. Tian, C. Du, C. Jin, Occupational exposure to benzene in China, Br. J. Ind. Med. 44 (1987) 192–195.
[31] S.-N. Yin, G.-L. Li, F.-D. Tain, Z.-I. Fu, C. Jin, Y.-J. Chen, S.-J. Luo, P.-Z. Ye, G.C. Wang, X.-C. Zhang, H.-N. Wu, Q.C. Zhong, A retrospective cohort study of leukemia and other cancers in benzene workers, Environ. Health Perspect. 82 (1989) 207–213.
[32] S.-N. Yin, R.B. Hayes, M.S. Linet, G.-L. Li, M. Dosemeci, L.B. Travis, Z.-N. Zhang, D.-G. Li, W.-H. Chow, S. Wacholder, W.J. Blot, The Benzene Study Group, An expanded cohort study of cancer among benzene-exposed workers in China, Environ. Health Perspect. 104 (Suppl. 6) (1996) 1339–1341.
[33] D.C. Braun (Ed.), Proceedings of the Symposium on Toxicology of Benzene and Alkylbenzenes, Industrial Health Foundation, Pittsburgh, PA, 1974.
[34] IARC, Benzene. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 29, International Agency for Research on Cancer, Lyon, France, 1982, pp. 93–148.
[35] S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffee, S.A. Pileri, H. Stein, J. Thiele, J.W. Vardiman, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, fourth edition, International Agency for Research on Cancer, Lyon, 2008.
[36] R. Baan, et al., A review of human carcinogens – Part F: Chemical agents and related occupations, The Lancet Oncology 10 (2009) 1143–1144.

Please cite this article in press as: M.G. Bird, et al., BENZENE 2009—Health effects and mechanisms of bone marrow toxicity: Implications for t-AML and the mode of action framework, Chem. Biol. Interact. (2010), doi:10.1016/j.cbi.2009.12.001