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International symposium: Recent advances in benzene toxicity

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Abstract

The symposium overview describes the recent succession of scientific meetings to further understand the adverse effects of benzene. It reviews the epidemiological evidence for hematological changes including leukemias and non-Hodgkins lymphoma, the progress in molecular biology and mechanism of action as well as ongoing studies in these fields. Various national occupational and community exposure monitoring data show that there has been significant progress in reducing benzene exposures globally. Biomarkers and biomonitoring have been used to provide information on biological plausibility, mode of action and susceptibility, and in the discrimination of co-exposures. Current research should help to further clarify cell type specificity, relationship to exposure, and the molecular elements involved in mechanism.

Keywords: Benzene; Leukemia; Lymphoma; Myelodysplastic syndrome; Toxicity; Carcinogenicity; Epidemiology; Exposure; Biomarkers; Biomonitoring; Genotoxicity; Hematological effects; Symposium

1. Introduction

A growing international awareness of the hazard presented by occupational exposure to benzene is evident in the successive evaluations of the International Agency for Research on Cancer between 1974 and 1986. In vol. 7 of the IARC Monographs on the Evaluation of Carcinogenic Risks of Chemicals to Man (1974), note was taken of the potential of occupational benzene exposure to result in damage to the hematopoietic system and to cause fatal acute poisoning under some conditions of occupational exposure. Numerous case reports of various forms of leukaemia, following protracted exposure to benzene were noted, but this evidence of possible causation was considered only suggestive, and bioassays of benzene for carcinogenicity to experimental animals were considered inconclusive at that time [1]. When benzene was next evaluated 7 years later, in 1981, the volume of published scientific literature had increased dramatically, and epidemiological evidence from analytical cohort and case–control studies now provided statistically significant associations between leukemia (predominantly myeloid) and occupational exposure to benzene and benzene-containing solvents [2]. No association was seen between benzene

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exposure and risk of other cancers. This association was consistent, but not always precisely defined (various leukemias were noted in different studies, including leukemias of myeloid, monocytic, erythroblastic and lymphocytic cell types). However, association between occupational benzene exposure and increased risk of leukemia in general was considered sufficient evidence of carcinogenicity to humans, despite limited evidence for carcinogenicity of benzene to experimental animals. When the most recent evaluation of benzene by IARC took place 5 years later, benzene was among the first substances classified by the newly established evaluation system as *carcinogenic to humans* (Group 1) [3].

Evidence for genotoxicity of benzene was documented in the 1986 IARC evaluation [3], but details of how this toxicity was mediated were unclear then, and the mechanisms of benzene toxicity to bone marrow continue to be a subject of active research today. A succession of conferences with published proceedings since 1986 document, the continuing challenge of fully understanding the adverse health effects of benzene.

2. Historical

Both for those scientists actively engaged in benzene research and others who may be peripherally involved, there has been a productive and exciting series of international symposia and a workshop on the topic of benzene. A symposium in 1988 [4], soon after the last of the three IARC evaluations, was followed by a workshop in Ottawa in 1991 [5], a second symposium Benzene ’95 [6], and now the symposium reported here, which was held in Munich in October 2004.

The 1998 symposium led with the proposal by Goldstein [7] that contrary to the concept of Occam’s razor being dull, i.e. that the simplest hypothesis is best, benzene toxicity is complex, involving more than one metabolite acting through more than one mechanism thereby producing more than one biological effect. In his preface to Benzene ’95 [8], Snyder noted that significant advances in molecular biology and hematology enabled a better understanding of the physiology of hematopoiesis and the effects of benzene on bone marrow. These meetings have provided the opportunity to review and present ongoing research. At Ottawa, there was focus on physiologically based pharmacokinetics and biomathematical modeling as well as reporting on the ongoing epidemiology study of the National Cancer Institute in China. In this special issue, the 2004 Munich symposium reports on further exposure and clinic-based population studies in Shanghai, China, as well as advances in the state-of-the-science relative to a mechanistic understanding of benzene toxicity.

Much of the new data derive from recent advances in molecular biology. Occam’s razor may be blunt, but is no doubt getting sharper as exciting new developments advance the field of benzene toxicology. We may well be still limited in our thinking about the pathways involved in benzene’s mode of action as a toxicant, but the research in progress reported in this special issue is a testimony to exciting developments and points to significant progress in our understanding of benzene-induced leukemogenesis. With the recognition that further advances in our knowledge of benzene toxicity are just around the corner, the next symposium should not be too far off.

3. Epidemiology

Benzene has long been known to cause leukemia, but there are still uncertainties involving disease specificity and concentration–response relationships. A review of prior published clinical and epidemiological studies shows that while there is clearly a causal relationship between benzene exposure and acute myelogenous (myeloid) leukemia (AML) and possibly chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL) as well, there are sparse data regarding acute lymphocytic leukemia (ALL). There is a paucity of data by cell type. Although there are 10,000–12,000 cases of AML per year (all causes) in the USA, this is still a relatively rare disease. Earlier imprecision in the definition of cell types is being rectified in current studies; older studies, where this was not determined, are of limited value.

Benzene effects are predominately on the myeloid cell lineage. Benzene-poisoning cases offer insight into disease progression; while repeated exposures lead to hematological effects, it is also true that risk decreases with time after last exposure. Following these cases offers an opportunity to explore the enigma of latency.

Lymphomas (which are lymphoid neoplasms differing from leukemias of the same cell type only by their clinical presentation in a given case) may have a differ-

ent mechanism. A possible causal association of benzene exposure with non-Hodgkin lymphoma (NHL) still remains an open question, but the recent Health Watch case control study of Australian Petroleum company workers showed no association with NHL or multiple myeloma.

4. Exposure assessment

Currently, the importance of peak exposure on benzene toxicity remains to be answered. That is, it is not known whether the area under the curve or saturation of uptake and/or metabolism plays the greater role in benzene toxicity. There has been considerable progress in reducing benzene exposures in China and in Korea, where exposures have traditionally been higher. Sources for benzene exposure used in recent or ongoing case control studies include a data base of 50,000 + benzene measurements maintained by the Shanghai Municipal Institute of Public Health Supervision ((IPHS), district IPHS, Chinese medical journals and measurements at factories. In most of the Western world, typically most of the occupational exposures are currently at or below 3.25 mg/m^3 (1 ppm). While benzene levels in Eastern countries have been higher, these have been significantly reduced in recent years, with medians in China reducing from 270 mg/m^3 in 1981–1985 to 48 mg/m^3 in 1996–2000. Benzene exposures occur during the production of leather products, electronic devices, machinery, shoes and sports equipment. In Korea, while exposures are now generally less than 1 ppm, workers can be exposed to 40–50 ppm over short periods. These benzene air measurements have been correlated with urinary measurements of *trans*-, *trans*-muconic acid (ttMA). In Thailand, ambient air levels have also been assessed as 65 and 140 ppb in factory workers and service station attendants compared with 8 ppb in controls, the latter being in line with the 15 ppb found in environmental exposures in Europe. Smoking is a significant source of benzene exposure in both occupational and non-occupational groups.

5. Biomarkers and biomonitoring

The use of biomarkers as surrogates and/or causal intermediate endpoints offers to provide information on biological plausibility, mode of action and susceptibility and on exposure assessment including discrimination of confounding exposures. Urinary biomarkers of benzene metabolism, such as *S*-phenylmercapturic acid (SPMA) and *trans*-, *trans*-muconic acid have been used successfully for biomonitoring of benzene exposures as low as 0.1 ppm, with SPMA ($t_{1/2}$ elimination of 9 h and background of 5 μg/g) being the more sensitive. Urinary benzene is the most discriminatory biomarker and showed a relationship with airborne benzene at all levels of exposure studied including 0.1 ppm benzene. Hydroquinone and phenol are not good markers below 5 ppm.

Measurement of protein adducts has shown a non-linear rate of adduct production with saturation above 50 ppm. Exposures below saturation may produce proportionately more biologically relevant metabolites, with peak exposures being less important in this respect than cumulative exposures. Previous molecular cytogenetic markers have shown benzene-induced aneuploidy in metaphase cells by fluorescent in situ hybridization (FISH) assay but not in interphase. The use of OctoChrome FISH allows the examination of many more metaphases and permits screening for chromosomal changes in all 24 human chromosomes. The findings suggest that chromosomes 5 and 7 are more sensitive to loss than other chromosomes following exposure to benzene metabolites. It is not known if the induced aneuploidy is random or selective, nor is it known which metabolite(s) are responsible for this. Current studies of protein expression patterns from sera of shoe factory workers show that “omic” technologies may have significant potential to generate novel biomarkers of exposure and response to benzene.

Review of benzene-poisoning cases indicates that structural cytogenetic abnormalities may occur relatively later in the development of myelodysplastic syndrome (MDS) and acute myeloid leukemia. Also, a long-standing bone marrow dysplasia is observed in the absence of changes in peripheral blood; MDS may have been under-reported.

Cases examined included exposures to 200–500 ppm with trisomy 8, 5q−, −7 and 7q− reported. The different results obtained from the use of the same therapy to treat aplastic anemia in these cases suggests that more than one type of benzene-induced aplastic anemia exists. This can be clarified from the use of biomarkers to identify different types of aplastic anemia.

6. Co-exposures

Both past and current epidemiology studies in China clearly showed that there is significant co-exposure to other agents. This occurs both occupationally, such as exposure to toluene along with benzene in glues used in shoe making, and also domestically by, for example, smoking or exposure to pesticides. Leather bag manufacturing can involve exposures of benzene up to 2040 mg/m^3 with co-exposures of 949 mg/m^3 toluene and 8580 mg/m^3 hexane.

7. Mechanism of action

Benzene offers an example of metabolic multi-tasking, which may provide clues to its mode of action and toxicity. The cystolic proteins – quinone oxidoreductases – in progenitor and stem cells may have roles beyond their proposed detoxification of benzoquinones and may include protection from oxidative stress, and the maintenance of microtubule stability. Some benzene metabolites, specifically catechol and hydroquinone, initiate c-Myb signaling activity. This in turn leads to increased phosphorylation of c-Myb and increased production of reactive oxygen species (ROS).

Studies in NQO1 null mice have shown their natural propensity to develop myeloid hyperplasia, and substantially greater benzene-induced toxicity. Further studies also now indicate that benzene toxicity is regulated by a number of genetic pathways that affect the metabolism and DNA damage response pathways in the bone marrow.

Benzene consistently tests negative in short term mutagenicity assays yet produces cytogenetic effects in animals and humans with damage to chromosomes and/or to the process of chromosome division. Inhibitors of topoisomerase II (topo II), a nuclear enzyme important for DNA transcription and replication, disrupt this process and lead to chromosomal changes which may be aligned with AML rather than with lymphoma.

1,4-Benzoquinone is a strong topoisomerase II inhibitor and more potent than etoposide. While hydroquinone also inhibits topo II resulting in dose-dependent loss of cell viability and decrease in cell count, it produces changes different to that of the etoposide-like topo II inhibitors and hence may act through a different mechanism.

The therapy-related myeloid leukemias (t-AML) seen with chemotherapy or radiotherapy have distinct subtypes that have characteristic gene expression patterns but which have in common arrested differentiation in early progenitor cells. Establishing the molecular pathways involved in t-AML may facilitate the identification of selectively expressed genes that can be exploited for the development of targeted therapies. Benzene causes immunosuppression, but whether there are unique features to this as with corticosteroids, Epstein Barr virus, human immunodeficiency virus or cyclosporin is presently not known.

8. Summary

Significant progress has been made globally in reducing exposures to benzene. This is seen particularly in China, Korea and Thailand, as reported in this special issue. Although overall benzene exposure has been reduced, variances exist and as exposures become lower, the impact of these will be increasingly evident and important to assess.

There are still no simple answers to benzene-induced leukemia risk or to the disease process. Benzene effects seem to be predominately on the myeloid cell lineage, as is also seen with therapy-related leukemias. There are uncertainties involving the specific cell types of leukemias induced by benzene, and there are uncertainties regarding the concentration–response relationships. The possible association of non-Hodgkin lymphoma with benzene also remains open. It is recognized that the World Health Organization's revised classification of lymphoid and hematopoietic malignancies [9] needs to be considered, as distinctions between disease entities have changed, What were considered different clinicopathological entities 10 years ago are in some cases considered different manifestations of the same disease today. Tumor registries worldwide and new clinically based studies are now utilizing this classification, and their results may help to clarify the association and the cell-type specificity.

In terms of mode of action, topoisomerase II (topo II) inhibition is more aligned with leukemia than lymphoma, but there are differences in the action on topo II of benzene and its metabolites compared with other inhibitors. This observation casts doubt as to whether

benzene-induced leukemia can be solely attributed to this mechanism. More mechanistic research is needed to bring together, as a validated model, the various molecular elements currently being pursued.

A comparison of therapy-related AML shows that the risk of developing related leukemias declines after 8–10 years post-treatment even in the persistent presence of chromosomal abnormalities. There may be benefit in following benzene-poisoning cases to see if this is the case for benzene following cessation or reduction of benzene exposure. Since there are virtually no examples of a single genotoxic event directly causing AML, this implies that benzene-induced leukemia is also a multi-step process.

Occam's razor dictates simplicity. For benzene, the individual steps may be becoming more transparent, but their interrelationships and the scenarios necessary for the development of benzene-induced leukemia still appear to be complex.

References

[1] IARC, Benzene, IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, vol. 7, International Agency for Research on Cancer, Lyon, France, 1974, pp. 203–221.

[2] 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.

[3] IARC, Benzene, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Suppl. 7, International Agency for Research on Cancer, Lyon, France, pp. 120–122.

[4] (Proceedings of the Symposium) Benzene Metabolism, Toxicity and Carcinogenesis, Environ. Health Perspect. 82 (1989) 3–307.

[5] (Proceedings of the Symposium) Benzene Toxicity, Carcinogenicity, and Epidemiology, Environ. Health Perspect. 104 (1996) 1121–1146.

[6] D. Krewski, R. Snyder, P. Beatty, G. Granville, B. Meek, B. Sonawane, Assessing the health risks of benzene: a report on the benzene state-of-the-science workshop, J. Toxicol. Environ. Health 61 (2000) 307–338.

[7] B.D. Goldstein, Introduction: Occam's razor is dull to benzene metabolism, toxicity and carcinogenesis, Environ. Health Perspect. 82 (1989) 3–6.

[8] R. Snyder, Preface to benzene toxicity, carcinogenicity, and epidemiology, Environ. Health Perspect. 104 (Suppl. 6) (1996) 1121.

[9] E.S. Jaffe, N.L. Harris, H. Stein, J.W. Vardiman, Pathology and Genetics: Tumours of the Haematopoietic and Lymphoid Tissues. World Health Organization Classification of Tumours, International Agency for Research on Cancer, Lyon, France, 2001.