PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 8.

Benzene and haematological cancers

Bernard D. Goldstein and Martyn T. Smith

Introduction

Haematological cancers present an example of seeming discordance between epidemiological data and animal data, which is apparently resolved by mechanistic information. Known causes of haematological cancers in humans are ionizing radiation, chemotherapeutic agents, infectious agents such as human immunodeficiency virus (HIV), and occupational exposures to chemical agents such as formaldehyde and benzene. These agents are recognized as causes of acute myeloid leukaemia (AML), and all have been implicated in the causation of other forms of haematological cancers.

This chapter focuses on the issue of whether benzene can be considered to cause lymphoproliferative disorders in humans, as distinct from AML. Whereas epidemiological evidence establishes that benzene is a cause of human AML (IARC, 1987), long-term studies in experimental animals exposed to benzene generally have not indicated an increased risk of AML. In contrast, increased incidence of lymphoma has been readily evident in such studies, but the corresponding epidemiological evidence can be debated. However, recent mechanistic data, as well as information that has led to a reclassification of haematological neoplasms, are consistent with benzene being recognized as a cause of human lymphoproliferative disorders (Smith et al., 2007, Goldstein, 2010; Smith, 2010). Recent epidemiological evidence also strongly supports this notion (Bassig et al., 2015; Linet et al., 2015).

Studies in experimental animals

In 1974, IARC concluded that studies in experimental animals did not show evidence of carcinogenesis from exposure to benzene (IARC, 1974). Subsequently, results from two long-term studies of experimental animals exposed to benzene that did report cancer were published by a research group at New York University (NYU) in the USA (Snyder et al., 1980, cited in IARC, 1982), who exposed C57BL/6J and AKR/J mice to benzene by inhalation, and by Maltoni et al. (1989) in Bologna, Italy, who

exposed Sprague-Dawley rats by gavage. Both studies reported an increased incidence of lymphoma as well as a variety of other tumours. There are now at least seven studies, including long-term animal bioassays by the United States National Toxicology Program and by others, showing an increased incidence of lymphomas of various types (Huff et al., 1989; Farris et al., 1993; Huff, 2007; National Toxicology Program, 2007; Kawasaki et al., 2009).

In contrast, evidence that AML results from exposure to benzene has been difficult to obtain in experimental animals, and has generally been lacking in the same studies that readily showed an increase in the incidence of lymphatic cancers. In other NYU studies, 3 of 40 CD1 mice were found to have myeloproliferative disorders, and 1 of 40 Sprague-Dawley rats was found to have AML; the results are notable only because these disorders are rare in both species (Goldstein et al., 1982). The NYU group also noted an 8-fold increase in the number of early precursor cells in the bone marrow of CD1 mice exposed to benzene (Snyder et al., 1981). An increase in the number of haematopoietic progenitor cells was also noted by Cronkite et al. (1989), who also observed AML in CBA/Ca mice exposed to benzene. More recent inhalation studies by Kawasaki et al. (2009) found an increase in the incidence of AML in leukaemia-prone C3H/He mice exposed to benzene, particularly if they were *Trp53*-deficient. Thymic lymphomas and non-Hodgkin lymphoma (NHL) were also noted.

Epidemiological studies

The causal relationship between exposure to benzene and AML was accepted by the medical community long before cohort-based epidemiological studies provided unequivocal evidence. Despite several studies evaluating AML in shoe workers in Italy (Vigliani and Saita, 1964) and leather workers in Turkey (Aksoy, 1989), the relationship between benzene and AML required a conventional cohort study in a well-defined worker population (Infante et al., 1977) before causality was fully accepted.

Numerous epidemiological studies evaluating the relationship between exposure to benzene and different forms of lymphoma have yielded inconsistent results. Some studies suggest a relationship (e.g. Arnetz et al., 1991; Hayes et al., 1997; Fabbro-Peray et al., 2001; Kristensen et al., 2008; Wong et al., 2010), whereas others do not (e.g. Raabe et al., 1998; Divine et al., 1999; Bloemen et al., 2004).

Recent meta-analyses suggest an association of exposure to benzene with NHL, multiple myeloma, chronic lymphocytic leukaemia (CLL), and acute lymphoblastic leukaemia (ALL) (Infante, 2006; Smith et al., 2007; Steinmaus et al., 2008; Vlaanderen et al., 2011). However, at the Working Group meeting for Volume 100F of the *IARC Monographs*, the epidemiological evidence relating exposure to benzene to NHL or multiple myeloma was not deemed to establish causality. As discussed below, recent changes in diagnostic criteria complicate epidemiological evaluation of lymphoproliferative disorders, as does the recognition that the criteria will continue to evolve. Recently published epidemiological studies also conclude that exposure to benzene is associated with NHL and other lymphoproliferative disorders (Bassig et al., 2015; Linet et al., 2015).

Changing diagnostic criteria for haematological disorders

The classification of haematological disorders continues to evolve, including relatively recent modifications that have major implications for studying and understanding causality. These reclassifications are largely based on advances in understanding the pathological and molecular basis for haematological diseases, and on the development of assays that permit differentiation among the various haematological cell types. Myeloid leukaemias are no longer divided simply into AML and chronic myeloid leukaemia (CML). The French–American–British classification has eight distinct subtypes of AML (Bennett et al., 1989). The more recent World Health Organization (WHO) classification makes greater use of cytogenetic findings to subclassify AML (Swerdlow et al., 2008). Myelodysplasia has also been subdivided into various types, enabling recognition of the different pathways and rates of transformation to AML (Bennett et al., 1989; Swerdlow et al., 2008).

Similarly, the newer approaches to classifying NHL have divided this entity into more than 40 subtypes, while at the same time moving previously separate diseases into this general classification. NHL now includes CLL, ALL, and multiple myeloma, as well as the multitude of lymphocytic disorders that were formerly included within the NHL diagnostic rubric (Swerdlow et al., 2008). It should be noted that both CLL and ALL are no longer standalone diagnoses (Swerdlow et al.,

2008). This is not surprising, because clinicians have long known that there was little distinction in clinical course, prognosis, and response to chemotherapy between CLL and lymphomas with similar mature lymphocytes involving other organs but not blood, or between ALL and more aggressive lymphomas with similar primitive lymphocytes involving other organs but not blood. However, the ability to use molecular markers to build on this clinical insight permits reclassification.

Evidence for an overlap between myeloid and lymphoid cancers in humans

There are two lines of evidence that myeloid and lymphoid haematological cancers are closely related: (i) evidence that has led to the general acceptance that there is a common haematological stem cell, and (ii) clinical evidence based on the use of newer biological markers that show the overlap between the two types, which were previously considered to be separate. The clinical evidence includes recognition of the following: perhaps 10% of new cases of acute leukaemia have both lymphoblastic and myeloid characteristics; leukaemia that occurs in individuals with Down syndrome can be lymphoid or myeloid, as can leukaemia resulting from chemotherapy for cancer; and blast transformation of CML can be lymphoid rather than the more usual myeloid (Gassmann et al., 1997; Calabretta and Perrotti, 2004; Lee et al., 2009; Xavier et al., 2009).

Mechanism of benzene-induced haematotoxicity

Benzene, which has been a known cause of human bone marrow toxicity since the 19th century, produces its effects through metabolism to one or more haematotoxic metabolites. Like other well-established causes of AML, including ionizing radiation and chemotherapeutic agents, benzene has the ability to cause aplastic anaemia at high doses and a pancytopenic effect at lower doses, indicating toxicity to the pluripotent haematopoietic stem cell population responsible for the production of red blood cells, white blood cells, and platelets.

Lymphocytes are affected by benzene, as is evident from a decreased lymphocyte count in exposed workers (Goldstein, 1988; Rothman et al., 1996), and from the severe loss of function of lymphatic organs in experimental animals as a result of longer-term high-level exposure. Particularly pertinent to the mechanistic considerations with respect to whether benzene is a cause of lymphatic tumours is the finding in many studies of genotoxic effects in circulating lymphocytes of exposed humans or experimental animals (Vigliani and Forni, 1969; Forni, 1979; Zhang et al., 1996, 2002, 2005, 2011; Navasumrit et al., 2005). These effects are seen in bone marrow precursor cells and in circulating lymphocytes and include overt chromosomal abnormalities, translocations, deletions, and aneuploidy of several different chromosomes (Zhang et al., 2007, 2011, 2012). The evidence is consistent with exposure to benzene potentially affecting multiple sites within the genetic material of pluripotent stem cells.

Conclusions

Both IARC and the United States National Toxicology Program have in recent years changed their approach to classification of carcinogens to give additional emphasis to the role of mechanistic understanding. IARC now allows a chemical to be classified as *carcinogenic to humans* (Group 1) "when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity" (IARC, 2006).

In the case of benzene and lymphoproliferative cancers, there is clearly *sufficient evidence* in experimental animals and strong evidence in humans of a relevant mechanism of carcinogenicity. This evidence includes the recognition that the stem cell known to be at risk for benzene-induced AML is also responsible for lymphoproliferation, the well-known vulnerability of human lymphocytes to benzene toxicity, and the demonstration of increased chromosomal abnormalities in the circulating lymphocytes of workers exposed to benzene. It also includes the promiscuous DNA damage caused by benzene metabolites and the observation of aberrations in multiple chromosomes among workers who have been heavily exposed to benzene or who have haematological cancers attributable to benzene. These mechanistic findings bridging lymphoproliferative and myeloproliferative cancers appear to explain the seeming lack of congruence between epidemiological data and animal data for myeloid and lymphoid cancers observed with benzene, and with other agents.

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