Case Study: Cellular and Molecular Aspects of Benzene-Induced Bone Marrow Pathology

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Bone Marrow Diseases

- **Pancytopenia**: diagnosed by examining the peripheral blood; significant decreases in circulating blood cell populations due to effects in the bone marrow; functioning marrow
- **Aplastic anemia**: absence of cells in bone marrow; bone marrow replaced by fat
- **Myelodysplasia**: Group of disorders in which the bone marrow produces too few mature and/or functioning blood cells; begins with a change to a normal stem cell; pre-leukemia state?
- **Leukemia**: blood cell differentiation arrested at an immature state; immature cells continue to proliferate

Bone Marrow Toxicity and Drugs

- Because all lymphocytes, monocytes, neutrophils, and immune cells originate from BM, evaluation of the integrity of myelopoiesis and lymphopoiesis in this organ after immunomodulatory compounds is important to our understanding of how a compound ultimately alters immune responsiveness.
- Comprised immune responsiveness, caused by reduced hemopoiesis, can occur clinically. For example, residual hematopoietic damage has been reported after cytotoxic therapy for cancer.
- A common feature of this residual damage is reduced levels of hematopoietic precursors and circulating immune cells. In addition, immunosuppressive and immunomodulatory techniques, which alter hemopoiesis, are important in BM transplantation.
- Antiviral drugs, such as AZT (zidovudine), also have hematotoxic side effects that reduce circulating white blood cells and, paradoxically, further compromise immunity in susceptible individuals.

Discovery and Uses of Benzene

- Isolated and identified in 1825 by Faraday
- Used to decaffeinate coffee-Sanka
- Increases octane rating of gasoline; antiknocking properties; replaced lead in gasoline
- Intermediate in organic synthesis
- Currently used in hydraulic fractioning
History of Human Bone Marrow Pathology

- 1897: exposure related to a case of aplastic anemia
- 1914-1915: exposure related to immune effects
- 1928: first observation reported of benzene as a leukemogen
- 1971: leukemia in Turkish shoe factory workers
- 1977: leukemia in rubber workers in Akron

Chronic Health Effects of Benzene Exposure

Pancytopenia  
Aplastic Anemia  
Myelodysplasia  
Leukemia  

Odor threshold  
1 ppm

Relationship of benzene exposure to various hematological disorders

A. Causality Proven
- Pancytopenia: Aplastic Anemia
- Acute Myelogenous Leukemia and Variants (Including Acute Myelomonocytic Leukemia, Acute Promyelocytic Leukemia, Erythroleukemia)

B. Causality Suspected
- Chronic Myelogenous Leukemia
- Chronic Lymphocytic Leukemia
- Hodgkin’s Disease
- Paroxysmal Nocturnal Hemoglobinuria

C. Association Suggested But Unproven
- Acute Lymphoblastic Leukemia
- Myelofibrosis and Myeloid Metaplasia
- Lymphoma: Lymphocytic, Histiocytic
- Thrombocytopenia

Benzene Primary Metabolites

First Pass Effect in the Liver
Detoxification and Excretion (Toxicokinetics)
Bone Marrow Cell Toxicities (Toxicodynamics)
Table 2

Metabolic susceptibility factors in bromo toxicity.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Susceptibility pathway</th>
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</thead>
<tbody>
<tr>
<td>CYP2E1</td>
<td>Rapid metabolizer phenotype and SNPs</td>
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<tr>
<td>CYP4F3</td>
<td></td>
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<tr>
<td>NQO1*2</td>
<td>Heterozygous (decreased activity) and homozygous (null) GSH</td>
</tr>
<tr>
<td>NQO1*3</td>
<td>Reduced NQO1 activity</td>
</tr>
<tr>
<td>GSH</td>
<td>Enzymes regulating levels</td>
</tr>
<tr>
<td>EN</td>
<td>Enzymes regulating levels</td>
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<tr>
<td>Null variants in GSTT1 and GSTM1, GSTH variants with decreased activity</td>
<td></td>
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</tbody>
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For citations see text.
Schematic model depicting the inter-relationship of bone marrow macrophages with fibroblastoid stromal cells.
Animal Model Studies

Ah receptor and Hematopoiesis

- It is hypothesized that AhR expression is necessary for the proper maintenance of quiescence in HSCs, and that AhR down-regulation is essential for "escape" from quiescence and subsequent proliferation of these cells. This implicates the AhR as a negative regulator of hematopoiesis with a function of curbing excessive or unnecessary proliferation.
- This provides an important advantage by preventing the premature exhaustion of HSCs and sensitivity to genetic alterations, thus preserving HSC function and long-term multi-lineage generation over the lifespan of the organism.
- AhR dysregulation may result in the altered ability of HSCs to sense appropriate signals in the bone marrow microenvironment leading to hematopoietic disease.
In Vitro Cytogenetic Changes

Studies of patients occupationally exposed to benzene show a pattern of cytogenetic aberrations involving high frequency of loss of all or part of chromosomes 5 and/or 7 as well as trisomy 8. Using fluorescence in situ hybridization with chromosome-specific 5, 7 and 8 probes it was demonstrated that 42, 49 and 26 microM HQ induces monosomy 5, 7 and 8, respectively, in the human lymphoblast cell line GM09948. These results demonstrate for the first time that HQ induces a specific chromosome loss found in secondary MDS/AML. The pattern of chromosome 5 and/or 7 loss in benzene-induced MDS/AML is probably due to selective cell survival after HQ exposure rather than specific targeting of HQ for chromosomes 5 or 7.

Immunofluorescence Results

Control Cells

IF Results continued:

6 Hour 5 μM HQ exposure

Microarray Results: HQ

HQ

GSH

BQ-503

Quinone Reductase

Centromere Protein A - 1.46

Histone 1 - 1.68

DNA Helicase - 1.67

Topoisomerase II - 1.34

Centromere Protein F - 1.46

TPX2 - 1.36

HQ elicits a response characteristic of electrophilic stress and the structural prevention of cell division
Interleukin-3 (IL-3) and granulocyte/macrophage-colony-stimulating factor (GM-CSF) are responsible for maintaining survival and stimulating growth of early dormant hematopoietic progenitor cells (HPC). These cytokines exhibit extensive overlap, with GM-CSF supporting growth and differentiation of myeloid HPC.

A characteristic shared by a diverse group of leukemogens is the ability to act synergistically with GM-CSF to increase clonogenic response.

Previous studies have revealed that pretreatment of murine HPC with hydroquinone (HQ) but not phenol, catechol, or trans-trans-muconaldehyde results in a selective enhancement of GM-CSF but not IL-3-mediated clonogenic response. Pretreatment of murine bone marrow cells in vitro results in increased numbers of HPC dividing and forming colonies in response to GM-CSF but not IL-3.

HQ pretreatment of murine HPC did not induce either an up- or a down-regulation of GM-CSF receptors or any change in receptor affinity.

CD34+ cells, which represent between 1 and 5% of human bone marrow, contain virtually all clonogenic stem and HPC. Pretreatment of CD34+ cells (approximately 95% purity) with HQ also results in enhanced clonogenic response with GM-CSF but not IL-3. These findings suggest that an early step in chemical leukemogenesis may involve transient alterations in the regulation of cytokine response to GM-CSF.

Systems Biology Approaches

Hematotoxicity, a significant decrease in almost all blood cell counts, was identified as a phenotypic effect of benzene that occurred even below 1 ppm benzene exposure.

A significant decrease in the formation of progenitor colonies arising from bone marrow stem cells with increasing benzene exposure, showing that progenitor cells are more sensitive to the effects of benzene than mature blood cells, likely leading to the observed hematotoxicity.

Analysis of transcriptomics by microarray in the peripheral blood mononuclear cells of exposed workers, identified genes and pathways (apoptosis, immune response, and inflammatory response) altered at high (>10 ppm) and low (<1 ppm) benzene levels.

Serum proteomics by SELDI-TOF-MS revealed proteins consistently down-regulated in exposed workers. Preliminary epigenomics data showed effects of benzene on the DNA methylation of specific genes.