Arvinic-Induced Bone Marrow Toxicity: Ultrastructural and Electron-Probe Analysis

By John R. Feussner, John D. Shelburne, Stephen Bredehoeft, and Harvey Jay Cohen

A patient with severe arsenic poisoning that resulted in marked peripheral blood and bone marrow abnormalities, including megaloblastic erythropoiesis, experienced many of the previously reported hematologic complications of arsenic poisoning: leukopenia, granulocytopenia, absolute eosinophilia, and profound anemia. In this study we report an ultrastructural and electron-probe analysis of the bone marrow. Although megaloblastic anemia associated with arsenic poisoning has been described rarely, the presence of arsenic in the local bone marrow milieu has not been demonstrated previously. The ultrastructural features of arsenic-induced bone marrow toxicity are similar to those described in other dyserythropoietic states and include marked nuclear aberrations involving shape, chromatin distribution, and nuclear envelope. Using the technique of energy-dispersive x-ray analysis (electron probe) we demonstrated arsenic in bone marrow spicules; this supports the contention that arsenic can cause megaloblastic anemia. We suggest that this technique may be a useful tool in further studies that attempt to explore the mechanism of arsenic-induced hematologic toxicity. Finally, we suggest that arsenic has a direct toxic effect on DNA synthesis that results in marked disturbances of nuclear division. We recommend that the most appropriate screening procedure to evaluate possible arsenic poisoning is tissue arsenic measurements (hair and nails) rather than 24-hr urinary measurements.

Early medicinal uses of arsenic included therapy for pernicious anemia with Fowler's solution. However, in 1943 Limarzi found no salutary effect of arsenic in patients with pernicious anemia treated sequentially with Fowler's solution and "liver therapy." More recently, Westhoff and others have suggested that arsenic toxicity can cause megaloblastic anemia. Thus the role of arsenic in megaloblastic anemia has come full circle—from therapy for pernicious anemia to a potential cause of megaloblastic erythropoiesis.

Although arsenic toxicity produces a variety of hematologic abnormalities, a causal relationship to megaloblastic anemia remains unproved. In this report we describe a patient with severe arsenic poisoning resulting in marked anemia and peripheral blood and bone marrow abnormalities, including megaloblastosis, and we present an ultrastructural and electron-probe analysis of the bone marrow.

CASE REPORT

W.B., a 32-yr-old black Viet Nam veteran presented to the Durham V.A. Hospital with complaints of progressive painful sensations in both legs and inability to walk or rise from a sitting position. Although present for several months, his symptoms became especially severe 2–3 wk prior to presentation.

From the Divisions of General Internal Medicine and Hematology, Department of Medicine, and the Department of Pathology, Duke University Medical Center, and the Durham Veterans Administration Hospital, Durham, N.C.

Submitted August 4, 1978; accepted December 28, 1978.

Supported in part by the Durham Veterans Administration Hospital, Diagnostic Electron Microscopy Laboratory and by grant 805460 from the Environmental Protection Agency.

Address reprint requests to John R. Feussner, M.D., Ambulatory Care Section, Department of Medicine, Durham V.A. Hospital, Durham, N.C. 27705.

© 1979 by Grune & Stratton, Inc. ISSN 0006-4971/79/5305-0003$01.00/0

820 Blood, Vol. 53, No. 5 (May), 1979
Additional complaints included anorexia, nausea, vomiting, crampy midepigastric abdominal pain, and a 5-7-kg weight loss. Most recently, he noted scaling skin changes on his soles and palms. On admission, he was using no medications, and he disclaimed recent alcohol abuse.

The admitting physical examination revealed a chronically ill-appearing man. His skin was abnormal, with thick hyperkeratotic and desquamating areas confined to the palms and soles. Although not icteric, the mucous membranes were pale, and the tongue was papillated normally. A soft systolic ejection murmur was audible. The lungs and abdomen were normal. The patient was orientated and cooperative. There was bilateral dysmetria and dysdiadochokinesis. The hands and wrists were weak (3/5) without wristdrop. The anterior tibial and gastrocnemius muscles were weak (3/5) without footdrop. The patient could neither rise from a sitting position nor walk without assistance. His gait was unsteady, slapping, and wide-based. He was areflexic. Pain sensation to pinprick was absent in a stocking and glove distribution, and proprioception was diminished in his feet.

Laboratory data included hemoglobin 8.4 g/dl, PCV 26%, MCV 102 μm³/red cell, platelet count 255,000/cu mm., with a white blood cell differential of 47% neutrophils, 3% bands, 23% lymphocytes, 7% monocytes, 19% eosinophils, and 1% basophils. The total eosinophil count was 752/cu mm., the reticulocyte count was 1.6%, and the Westergren sedimentation rate was 129 mm/hr. Direct and indirect Coombs tests were negative, and serum protein electrophoresis, protime, and partial thromboplastin time were normal. Serum iron and total iron binding capacity were 37 mEq/dl (54-154 mEq/dl) and 177 mEq/dl (246-430 mEq/dl), respectively. Serum folate was 3.2 ng/ml (4-16 ng/ml), and serum B₁₂ was 759 pg/ml (200-900 pg/ml). Bone marrow determinations were performed on admission (May 19) and again on May 25 and June 6. Peripheral blood and bone marrow morphology will be described later. Bone marrow chromosome analysis demonstrated a normal karyotype. A 24-hr urinary lead level was zero (less than 24 μg/liter). Although no arsenic was present in a 24-hr urine collection, arsenic levels in hair and nails were extreme: 2.06 mg/g (0.001 mg/g).

During hospitalization, all gastrointestinal manifestations of arsenic toxicity resolved. Also, whereas the hyperkeratotic skin changes resolved, Mees lines developed on the 34th hospital day, thus suggesting that recent arsenic exposure ceased approximately 1 wk prior to hospitalization. The neurologic deficits progressed to include bilateral wristdrop and footdrop. The painful paresthesias seemed improved on discharge. The hematologic variables will be presented in detail in a following section. Finally, we could find no environmental source of arsenic to explain our patient's exposure.

MATERIALS AND METHODS

Heparinized bone marrow was gently layered over glass to allow direct visualization of spicules. Several of these were promptly fixed in 0.1-M sodium-cacodylate-buffered 3% glutaraldehyde overnight. Fixation in 1% osmium tetroxide in the same buffer was followed by ethanol dehydration and embedding in Epon. Thin sections stained with uranyl acetate and lead citrate were examined at 60 kV in a JEOL 100B transmission electron microscope. Several other spicules were not fixed in glutaraldehyde, but instead were placed directly on a metal stub and snap frozen in liquid nitrogen. The metal stub was transferred under liquid nitrogen into a Denton DV-502 freeze-etch unit and maintained at −196°C until a vacuum of 10⁻⁶ torr was obtained. The specimen was freeze-dried by slow warming over a 4-hr period to room temperature. Following a light carbon coating, the spicules were examined at 30 dV on an ETEC scanning electron microscope equipped with a Kevex Si (Li) energy-dispersive x-ray analyzer.

RESULTS

The peripheral blood film suggested heavy metal toxicity, with erythrocytes showing coarse basophilic stippling and multiple nucleated forms. Few macroovalocytes were seen, and no hypersegmented neutrophils were present. Absolute eosinophilia was present despite leukopenia. Platelet morphology was normal.

The initial bone marrow was striking in several respects. The biopsy revealed marked hypercellularity and erythroid hyperplasia. The erythroid and myeloid series demonstrated typical megaloblastic features (Fig. 1A). In addition, bizarre
Fig. 1. Representative light micrographs of Wright-stained bone marrow obtained on admission. (A) Erythroid precursors demonstrating typical megaloblastic features, with dissociation of cytoplasmic and nuclear maturation and a high nuclear/cytoplasmic ratio. Megaloblastic myeloid precursors are also present (X400). (B) Oil-immersion views of megaloblastic erythroid precursors, basophilic stippling, and bizarre karyorrhexis. Several cells are both karyorrhectic and megaloblastic (X 1000).

Karyorrhexis, coarse basophilic stippling, and numerous Howell-Jolly bodies were present (Fig. 1B). Approximately 40% of all myeloid cells counted were eosinophil precursors. Megakaryocytes were numerous, distinguished only by marked lobulation of their nuclei. Iron was abundant, and ringed sideroblasts were absent. The results of serial bone marrow examinations and other hematologic variables are listed in Table 1.

Electron photomicrographs of erythroblasts confirmed the presence of marked nuclear aberrations involving shape, chromatin distribution, and nuclear envelope (Fig. 2A–D). The majority of chromatin was heterochromatin. The cytoplasm of normoblasts showed rhotheocytotic invaginations along the plasma membrane in addition to similar intracytoplasmic vesicles. The membranes and vesicles were lined by ferritin-like granules. Occasional secondary lysosomes were seen within the cytoplasm. Mitochondria frequently exhibited ferruginous micelles in the matrix space.

Bone marrow spicules were examined by electron-probe analysis to demonstrate the presence of arsenic. Figure 3A demonstrates the K alpha arsenic peak in the patient’s marrow. This peak was absent from control regions of the stub lacking marrow spicules and from a control patient’s marrow (Fig. 3B). A peak at the L line of arsenic was also visible with longer counting times. The sensitivity of energy-dispersive x-ray microanalysis varies with different samples but is usually in the range of hundreds of parts per million. Most elements with atomic numbers greater than that of sodium can be distinguished from one another easily, especially
### Table 1. Serial Hematologic Variables

<table>
<thead>
<tr>
<th>Date</th>
<th>Hospital Day</th>
<th>Hemoglobin (g/dl)</th>
<th>Packed Cell Volume (%)</th>
<th>Reticulocytes (%)</th>
<th>WBC (10^9/liter)</th>
<th>Platelets (10^9/liter)</th>
<th>WBC Differential*</th>
<th>Westergren Sedimentation Rate (mm/hr)</th>
<th>Bone Marrow Morphology†</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/19</td>
<td>Admission</td>
<td>9.4</td>
<td>26</td>
<td>1.6</td>
<td>3.3</td>
<td>255</td>
<td>47</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>5/23</td>
<td>4</td>
<td>7.5</td>
<td>25</td>
<td>2.2</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/25</td>
<td>6</td>
<td>7.2</td>
<td>21</td>
<td>2.2</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/31</td>
<td>12</td>
<td>9.7</td>
<td>28</td>
<td>3.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/6</td>
<td>18</td>
<td>11.8</td>
<td>35</td>
<td>3.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/13</td>
<td>25</td>
<td>12.3</td>
<td>38</td>
<td>2.4</td>
<td>8.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/2</td>
<td>34</td>
<td>12.8</td>
<td>39</td>
<td>2.4</td>
<td>6.0</td>
<td>556</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/7</td>
<td>39</td>
<td>13.5</td>
<td>41</td>
<td>7.5</td>
<td>330</td>
<td>47</td>
<td>21</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>7/14</td>
<td>46</td>
<td>13.0</td>
<td>39</td>
<td>6.7</td>
<td>265</td>
<td>47</td>
<td>43</td>
<td>43</td>
<td>2</td>
</tr>
<tr>
<td>7/22</td>
<td>54</td>
<td>12.3</td>
<td>37</td>
<td>2.3</td>
<td>5.0</td>
<td>49</td>
<td>43</td>
<td>38</td>
<td>5</td>
</tr>
</tbody>
</table>

*PMN, polymorphonuclear leukocytes; JUV, juvenile; Lymph, lymphocytes; Mono, monocytes; Eos, eosinophils; Baso, basophils.
†M:E, myeloid:erythroid ratio.
Fig. 2. (A) Low-magnification electron micrograph of bone marrow exhibiting numerous abnormal erythroblasts (×2050). (B) Serial section of the "binucleate" cell seen in section A. This cell is, in fact, not binucleate; the two lobes of the nucleus are connected by a chromatin bridge. One siderosome is present in this cell (bottom right), and ferruginous micelles are visible in the mitochondrial matrix of an adjacent erythroblast (top right) (×6900). (C) Another erythroblast present in section A exhibiting marked irregularity of nuclear contour; note the two protrusions of euchromatin above (×9100). (D) This erythroblast exhibits a Howell-Jolly body and a mitochondrion with ferruginous micelles in the matrix space (×13,900).
ARSENIC-INDUCED BONE MARROW TOXICITY

Fig. 3. (A) X-ray spectrum of the freeze-dried marrow. The long vertical line indicates the correct position for the K alpha peak of arsenic seen below. The number of counts is displayed on the ordinate, and the energy of the x-rays is displayed on the abscissa. Two large peaks for copper (from the specimen support) are visible at the left. Between them and the arsenic peak there is a peak for platinum (from the carbon coating). (B) X-ray spectrum of a control region of the stub devoid of marrow. No arsenic peak is seen at the energy that corresponds to the K alpha peak of arsenic. As in section A, two large peaks for copper are visible at left. The microscope conditions are identical in sections A and B, including the total number of counts collected (778,000).

when the peak of interest lies in a region of the spectrum devoid of peaks from other elements (compare Figs. 3A and 3B). The usual resolution obtainable with energy-dispersive x-ray microanalysis is approximately 150 eV.9

DISCUSSION

Our patient manifested all of the common clinical features of arsenic poisoning—gastrointestinal, cutaneous, and neurologic symptoms and signs.17 In addition, laboratory confirmation of the clinical findings was unquestionable, given the 2.06 mg/g of tissue arsenic level. The negative urine determination emphasizes the potential lack of sensitivity of that test, suggesting that it is an inadequate screening test for arsenic exposure.

Our patient also experienced many of the previously reported hematologic complications of arsenic poisoning (Table 2). The bone marrow exhibited the marked red blood cell nuclear karyorrhexis classically described in arsenic intoxication and not seen to this degree in conditions other than heavy metal intoxication or the hereditary multinuclearity syndromes.3,18 Mealoblastic erythropoiesis has been

<table>
<thead>
<tr>
<th>Table 2. Hematologic Effects of Arsenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxic Manifestation</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Anemia</td>
</tr>
<tr>
<td>Uncertain cause</td>
</tr>
<tr>
<td>Aplasia</td>
</tr>
<tr>
<td>Megaloblastic—normal B12, folic acid</td>
</tr>
<tr>
<td>Megaloblastic—low folic acid</td>
</tr>
<tr>
<td>Leukopenia (usually accompanied by granulocytopenia)</td>
</tr>
<tr>
<td>Eosinophilia</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
</tr>
</tbody>
</table>
described only twice previously in arsenic intoxication,⁴ ⁵ although Kyle⁶ also referred to occasional “megaloblastoid forms” in one of his patients. In our patient, the vitamin B₁₂ level was normal, whereas the folate level was in the indeterminate range. Waxman and Schreiber¹⁹ and others²⁰ ²¹ have suggested that only folate levels of 0–3 ng/ml, as measured by radioassay, represent folic acid deficiency. Our patient had a level of 3.2 ng/ml. Moreover, serial bone marrow determinations documented resolution of the megaloblastic anemia within 3 wk, despite no dietary changes or specific replacement therapy. Thus, our patient appears to have megaloblastic anemia produced directly by arsenic poisoning.

An ultrastructural description of arsenic-induced bone marrow abnormalities has not been reported previously. However, unique ultrastructural features of dyserythropoiesis in aplastic anemia have been described by Frisch et al.²² Several of these features were present in our patient, including the marked aberration of nuclear shape and nuclear envelope. Both Frisch’s patient and ours demonstrated binucleated and multinucleated cells, internuclear chromatin bridges, irregular nuclear shapes, and several anomalies of the nuclear membrane. Frisch suggested that abnormalities of nuclear shape, impaired mitoses, and multinuclearity may be directly referred to a defective nuclear envelope. He noted close proximity of the nuclear envelope to the endoplasmic reticulum (ER), also noting that the ER is the primary site for many detoxification reactions. Since erythroblasts have little ER, he postulated that this function might be performed by the nuclear envelope, thus exposing it to toxic agents. If this postulate were proved, it could also identify the nuclear envelope as the site of injury in arsenic poisoning. This could explain the light and electron microscopic findings so characteristic of this disease.

Previous reports have not demonstrated arsenic in the local bone marrow environment, probably because of technical limitations. Our own experience²³ ²⁴ and the experience of others²⁵ suggested that examination of bone marrow spicules could be undertaken using the technique of energy-dispersive x-ray analysis. Our demonstration of arsenic within the bone marrow by this method adds credence to the contention that arsenic causes the observed bone marrow abnormalities. This report suggests that the technique may be useful in further studies that attempt to explore the mechanism of arsenic-induced hematologic toxicity.

In an effort to explain the cytotoxic and mutagenic effects of arsenic, Petres et al. studied the effects of inorganic arsenic on DNA synthesis in human lymphocyte cultures.²⁶ They demonstrated a direct toxic effect of arsenic on DNA synthesis in human lymphocytes that resulted in marked disturbances of nuclear division even in the presence of relatively low doses of arsenic. Both ³H-thymidine labeling index and mitotic index were considerably reduced in the presence of sodium arsenate. The types of nuclear changes described in these in vitro studies are quite similar to the changes seen in the red cell precursors in the bone marrow of our patient. This, together with our direct demonstration of arsenic in the bone marrow milieu, suggests that arsenic might act in an analogous manner in vivo to produce the megaloblastosis and karyorrhexis.

Finally, it does not seem prudent to evaluate all patients with megaloblastic anemia for arsenic poisoning. However, any suggestion of nuclear karyorrhexis should raise this diagnostic possibility, and appropriate studies (nail and/or pubic hair arsenic levels) to evaluate arsenic poisoning should be performed.
ACKNOWLEDGMENT

The authors are grateful to Dr. Joseph Moore, who collected the initial blood and bone marrow samples. We appreciate the skilled assistance of Dr. Peter Ingram of the Research Triangle Institute and his help with the microprobe study, as well as the technical assistance of Dr. Joachim Sommer, Walter Fennel, Mike Hale, and Jessie Calder. We thank Cynthia L. Dix for excellent secretarial assistance.

REFERENCES

8. Webster SH: The lead and arsenic content of urines from 46 persons with no known exposure to lead or arsenic. Public Health Rep 56:1953, 1941
12. McCarthy FP, Wilson R: The blood dyscrasias following the arsphenamines. JAMA 99:1557, 1932
13. Dodd K, Williamson SJ: Severe granulocytic aplasia of the bone marrow. JAMA 90:663, 1928
Arsenic-induced bone marrow toxicity: ultrastructural and electron-probe analysis

JR Feussner, JD Shelburne, S Bredehoeft and HJ Cohen