Anticonvulsant-Induced Aplastic Anemia: Increased Susceptibility to Toxic Drug Metabolites In Vitro

By William T. Gerson, David G. Fine, Stephen P. Spielberg, and Lyle L. Sensenbrenner

A 53-yr-old man sequentially developed aplastic anemia from phenytoin and carbamazepine. Both compounds undergo metabolism to potentially toxic arene oxide intermediates. We tested the hypothesis that the patient’s adverse reactions were due to a defect in detoxification of such metabolites by challenging his peripheral lymphocytes with drug metabolites generated by a murine hepatic microsomal system in vitro. The patient’s cell viability was normal in the absence of drugs. However, his cells showed greater toxicity from both phenytoin and carbamazepine metabolites than did controls. Toxicity was dependent on microsomes and NADPH. Intermediate toxicity was noted in cells from the patient’s mother. The results provide the first evidence for a role of arene oxide drug metabolites in aplastic anemia in humans and suggest that enhanced susceptibility to toxicity may be based on an inherited abnormality in metabolite detoxification.

Despite intensive recent research, the pathophysiology of drug-induced aplastic anemia remains obscure. Demonstration of a relationship between a specific drug and bone marrow toxicity is generally based on temporal association, often in the face of multiple drug use, and rechallenge with the suspected offending agent is medically and ethically unjustified. Bone marrow aplasia and hepatotoxicity rarely occur during therapy with phenytoin or carbamazepine. Both compounds undergo oxidative metabolism with the formation of potentially toxic arene oxide intermediates. If not detoxified, arene oxide metabolites of a variety of xenobiotics can bind covalently to cell macromolecules and cause genetic (mutations) and cytotoxic damage, and, by acting as hapten, lead to secondary immune reactions.

Individual susceptibility to idiosyncratic drug reactions from reactive metabolites could result from their increased production and/or decreased detoxification. In inbred mouse strains, genetically determined inducibility of cytochrome P-450 mono-oxygenases, which mediate arene oxide formation, influences the likelihood of hepatic and bone marrow toxicity on exposure to polycyclic hydrocarbons. In the case of the anticonvulsants, arene oxide metabolites of drugs with relatively high incidences of hepatotoxicity and aplastic anemia in clinical use (e.g., mephentoin and phenacemide) are more toxic to human lymphocytes in vitro than are metabolites of drugs with only rare cytotoxic complications (e.g., phenobarbital and phenytoin). Further, we have shown that susceptibility of some patients to phenytoin hepatotoxicity results from an inherited defect in phenytoin arene oxide detoxification.

We have been developing an in vitro assay for studying the role of reactive metabolites in human idiosyncratic drug reactions and aiding in the etiologic diagnosis of such reactions. In the assay, metabolites of drugs generated by a mouse hepatic microsomal system are targeted at human lymphocytes, and individual differences in susceptibility to toxicity are examined. Lymphocytes were chosen as easily obtained human cells that contain cell defenses against electrophilic metabolites, including glutathione and epoxide hydrolase. We now describe a patient with a history of two episodes of aplastic anemia, the first thought to be related to phenytoin, and the second to carbamazepine. The assay system has allowed in vitro challenge of the patient’s cells and cells from his mother by these two anticonvulsants and their metabolites, and exploration of the role of altered susceptibility to toxic drug metabolites in human drug-induced bone marrow hypoplasia.

CASE REPORT

In January 1981, a 53-yr-old white male farm laborer was transferred to The Johns Hopkins Hospital for the evaluation of pancytopenia and fever. His past medical history was remarkable for a chronic seizure disorder secondary to sustaining a depressed fracture of the left temporal bone at age 15 yr. Seizures had been moderately well controlled with phenytoin and phenobarbital for several months when, at age 37, he was hospitalized for dyspnea and easy fatigability approximately 1 mo after his dose of phenytoin had been increased from 300 mg to 500 mg/day. Laboratory data included a hematocrit of 14%, hemoglobin of 5.2 g/dl, reticulocyte count of 0.2%, leukocyte count of 2700/cu mm (segmented neutro-
cultures were positive for Escherichia coli. There was no indication of tumor or granuloma. Blood adhesion (nadir 8000/cu count showed a hematocrit of I weeks. At discharge, hematologic values were: hematocrit, erythroid and myeloid elements and only rare megakaryocytes. The liver and spleen were not enlarged. A complete blood biopsy showed a markedly hypocellular marrow with reduction of all hematopoietic elements with no evidence of leukemia or pernicious anemia. The diagnosis was made of "unregenerative anemia" compatible with a drug-induced hypoplasia of the marrow. Phenytoin was discontinued, and the patient was begun on primidone. He made an uneventful recovery and was discharged after 14 days with a hematocrit of 26%, hemoglobin of 9.5 g/dl, leukocyte count of 6500/cu mm (segmented neutrophils 62%, lymphocytes 36%, monocytes 2%), and a reticulocyte count of 0.3%.

The patient's hematologic status remained normal while taking primidone. In June 1980, his medication was changed to carbamazepine. With continued poor seizure control, primidone was re-instituted in October 1980, and the two drugs were continued together.

In January 1981, the patient noted lethargy, intermittent abdominal pain, and malaise. He was referred to The Johns Hopkins Hospital, where on admission he was found to be pale, febrile, and in moderate respiratory distress. Physical examination was remarkable for psoriatic lesions over the lower extremities, right upper quadrant tenderness with guarding, bright red blood per rectum, and disorientated mental status. There was no jaundice, petechiae, or ecchymoses. The liver and spleen were not enlarged. A complete blood count showed a hematocrit of 15.5%, hemoglobin of 5.5 g/dl, leukocyte count of 374/cu mm (segmented neutrophils 22%, band forms 12%, metamyelocytes 4%, lymphocytes 46%, monocytes 12%, basophils 2%, atypical lymphocytes 2%), and a platelet count of 11,000/cu mm. The SGOT was 615 IU/liter and the SGPT was >195 IU/liter; total bilirubin and alkaline phosphatase were normal. ANA, RF, hepatitis screens, RPR, and direct Coombs' were all negative. Total complement, and C3 and C4 were normal. A bone marrow biopsy showed a markedly hypocellular marrow with reduction of erythroid and myeloid elements and only rare megakaryocytes. There was no indication of tumor or granuloma. Blood cultures were positive for Escherichia coli.

The aplastic anemia was believed to be secondary to carbamazepine. Anticonvulsants were discontinued, and the patient was placed in protective isolation and treated with antibiotics. His pancytopenia necessitated repeated transfusions of packed red blood cells and platelets. Recovery of leukocyte counts began quickly (nadir 374/cu mm on admission, 3500/cu mm by hospital day 9), but platelet counts remained low during the entire admission (nadir 8000/cu mm on hospital day 10). SGOT and SGPT returned to normal over several weeks. At discharge, hematologic values were: hematocrit, 29.4%; hemoglobin, 10.2 g/dl; leukocyte count, 4500/cu mm (segmented neutrophils 69%, band forms 1%, lymphocytes 24%, monocytes 6%); and platelet count, 54,000/cu mm. The patient experienced no seizures while hospitalized and was discharged without anticonvulsants.

**MATERIALS AND METHODS**

The in vitro drug metabolite toxicity assay has been previously reported in detail.12 Lymphocytes were prepared from whole blood from the patient, his mother, and normal volunteers using Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ). The patient's cells were studied 1 day prior to his discharge from the hospital and 18 mo after full recovery. Cells were suspended in a Hepes-buffered medium (15 mM Hepes, pH 7.4; NaCl, 125 mM; KCl, 6 mM; MgSO₄, 1.2 mM; NaH₂PO₄, 1 mM; CaCl₂, 1 mM; and glucose, 10 mM) to yield 10⁶ cells/reaction. Hepatic microsomes were prepared from phenobarbital pretreated (60 mg/kg i.p. for 3 days) National Institutes of Health General Purpose Swiss Mice [N-GP(SW)]. A quantity of 0.5 mg of microsomal protein was incubated with the lymphocytes at 37°C for 2 hr along with 0.6 mM NADP, 2.4 mM glucose-6-phosphate, 2 U glucose-6-phosphate dehydrogenase, and varying drug concentrations, the latter added in dimethylsulfoxide (final concentration 5%). Cells were collected by centrifugation and resuspended in Hepes-buffered medium containing 5 mg/ml of albumin. Incubations were continued at 37°C for 16 hr and aliquots then taken for trypan blue dye exclusion. In previous studies, dye exclusion correlated well with other indices of cell damage, including lactic dehydrogenase release from the cells and loss of the ability to respond to concanavalin-A blastogenesis.13

**RESULTS**

When the full microsomal drug metabolizing system was included in the incubation mixture, phenytoin caused a dose-dependent increase in cell death in the patient's lymphocytes (Fig. 1). Over the concentration range studied (equivalent to 8–32 µg/ml), no toxicity was seen in lymphocytes from 25 control subjects, including 3 patients chronically exposed to phenytoin without side effects. (At higher concentrations of phenytoin metabolites, normal cells do exhibit toxicity.)9 In the case of carbamazepine, there was dose-dependent (7.5–30 µg/ml) toxicity of metabolites in normal cells. However, the patient's cells showed markedly increased toxicity.

![Fig. 1. Toxicity of phenytoin and carbamazepine to patient (O—O) and control (●—●) lymphocytes in the presence of the microsomal drug-metabolizing system. Values represent the mean of 2 determinations for patient cells; controls include duplicate determinations on 25 individuals (22 normal and 3 patients with seizures treated with phenytoin without adverse reactions) for phenytoin, and 4 normal controls for carbamazepine. Control values represent mean ±1 SEM. Horizontal lines represent mean percent dead cells in the presence of the microsomal system, omitting both drugs, with 1 SEM shown on the left. The patient's baseline percent dead cells did not differ from controls.](image-url)
When the patient's cells were re-examined 18 mo after his full recovery, comparable results were obtained with 125 μM phenytoin and carbamazepine (Table 1). The patient's cells showed a much smaller increase in cell death from phenobarbital metabolites. Cells from the patient's mother exhibited intermediate responses to metabolites of phenytoin and carbamazepine and no toxicity from phenobarbital.

Baseline viability by trypan blue dye exclusion of freshly prepared patient and control cells (patient 96.3%, control 96.2%) in the first series of experiments, and of patient, maternal, and control cells 18 mo later (patient 95.3%, mother 96.3%, control 95.8%) were comparable. Similarly, there was no difference in viability of patient, maternal, and control cells following incubation with microsomes and NADPH-generating system in the absence of the anticonvulsants (Table 1). In the initial experiments, omission of the NADPH-generating system, or microsomes and NADPH, abolished the toxicity of the anticonvulsants.

**DISCUSSION**

While the etiology of drug-induced aplastic anemia remains obscure, toxic, reactive metabolites of a variety of compounds may play a crucial role. Toxicity of electrophilic metabolites may result from covalent binding of the intermediates to cell macromolecules. Covalent binding of metabolites could lead to bone marrow toxicity through a variety of potential mechanisms (Fig. 2). Direct stem cell toxicity or mutation on immunologic processes involving formation of haptens or damage to lymphocytes with critical functions in hematopoiesis could occur.

Steady-state levels of electrophilic metabolites available to interact with cell macromolecules and produce toxicity are dependent both on their rates of production and detoxification. Susceptibility to bone marrow toxicity from polycyclic hydrocarbons in mice is related to genetic differences in inducibility of oxidative enzyme systems in liver and bone marrow that convert the compounds to arene oxides. Individual variation in response to potential electrophilic drug metabolite toxicity in man is complex and may depend on multiple genetic loci that regulate the production of metabolites.

**Fig. 2.** Postulated pathways of drug metabolism to arene oxide intermediates and the role of arene oxides in the pathogenesis of aplastic anemia. "R" represents the remainder of the phenytoin or carbamazepine structures.

**Table 1. Anticonvulsant Toxicity to Patient, Maternal, and Control Lymphocytes In Vitro**

<table>
<thead>
<tr>
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<th>Phenytoin</th>
<th>Carbamazepine</th>
<th>Phenobarbital</th>
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<tr>
<td>+ Microsomes</td>
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<td>+ NADPH</td>
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<tr>
<td>Control (5)</td>
<td>5.1 ± 0.5</td>
<td>5.7 ± 0.5</td>
<td>11.2 ± 0.7</td>
<td>5.5 ± 0.6</td>
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<tr>
<td>Patient (3)</td>
<td>5.0 ± 0.2</td>
<td>21.2 ± 0.5*</td>
<td>24.6 ± 0.4*</td>
<td>9.3 ± 0.6§</td>
</tr>
<tr>
<td>Mother (3)</td>
<td>5.1 ± 0.2</td>
<td>12.7 ± 0.5†</td>
<td>14.0 ± 0.8†</td>
<td>5.6 ± 0.7</td>
</tr>
<tr>
<td>- Microsomes</td>
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<td>- NADPH</td>
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<tr>
<td>Patient (2)</td>
<td>5.3</td>
<td>5.0</td>
<td>5.2</td>
<td>—</td>
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<tr>
<td>- Microsomes</td>
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<td>- NADPH</td>
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<tr>
<td>Control (3)</td>
<td>4.5 ± 0.3</td>
<td>5.3 ± 0.2</td>
<td>5.3 ± 0.4</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>Patient (2)</td>
<td>4.8</td>
<td>4.8</td>
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Cells were incubated in the presence or absence of microsomes and/or the NADPH-generating system, as indicated, with 125 μM of the anticonvulsants. Numbers in parentheses indicate the number of control subjects or replicate samples for the patient and mother. Results are expressed as mean ± SEM percent dead cells.

*Different from control and mother, p < 0.001, Student's t test.
†Different from control, p < 0.001.
‡Different from control, p < 0.05.
§Different from control, p < 0.01; from mother, p < 0.02.
the metabolites, their detoxification, and cell repair processes (Fig. 2). The approach used in this study, employing human lymphocytes as target cells for metabolites, was designed specifically to examine variation in cell defenses against reactive metabolites in the etiology of toxic drug reactions.

Our patient’s clinical course is highly suggestive of two episodes of drug-induced bone marrow toxicity, the first associated with phenytoin, the second with carbamazepine. He tolerated phenobarbital or primidone for long periods of time without hematologic or hepatic toxicity. The in vitro results correlate well with the clinical findings. Thus, the patient’s cells exhibited markedly increased toxicity from phenytoin and carbamazepine in the presence of the microsomal drug-activating system. The response to phenobarbital was significantly less and only slightly above baseline. Similar results were obtained immediately after the patient’s recovery and 18 mo later, suggesting that the in vitro phenomena were not secondary to the acute disease process. Furthermore, cells from the patient’s mother showed intermediate toxicity from phenytoin and carbamazepine and a normal response to phenobarbital. The abnormality in handling of drug metabolites thus appears to be inherited, and the familial pattern of response to different drugs consistent. Such family studies help confirm the diagnostic impression of a specific drug etiology of the patient’s symptoms and establish a pharmacogenetic basis for the drug reaction.

Toxicity of the anticonvulsants to the patient’s cells was dependent on microsomes and an NADPH-generating system. In previous studies, inhibition of the enzyme epoxide hydrolase, a major pathway for arene oxide detoxification, enhanced the toxicity of both compounds in normal cells, while addition of purified enzyme epoxide hydrolase, a major pathway for arene oxide detoxification, to the medium abolished toxicity. In vitro phenomena were not secondary to the acute disease process. Furthermore, cells from the patient’s mother showed intermediate toxicity from phenytoin and carbamazepine and a normal response to phenobarbital. The abnormality in handling of drug metabolites thus appears to be inherited, and the familial pattern of response to different drugs consistent. Such family studies help confirm the diagnostic impression of a specific drug etiology of the patient’s symptoms and establish a pharmacogenetic basis for the drug reaction.

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In previous studies, we have shown that cells from three patients with phenytoin hepatotoxicity exhibited increased damage from phenytoin arene oxides in vitro. Intermediate responses were noted in parents’ cells, and the responses in siblings further suggested an autosomal recessive defect in arene oxide detoxification. Several features distinguish the present patient from those previously reported with phenytoin hepatotoxicity. The patient did have hepatic as well as bone marrow damage from anticonvulsants. However, he developed aplastic anemia as opposed to leukocytosis, eosinophilia, and “pseudolymphoma” seen in most patients with hepatotoxicity from phenytoin. Target tissue specificity of toxicity on exposure to compounds requiring metabolic activation to reactive metabolites may be determined by genetically controlled relative activities of critical enzyme pathways in different tissues and by routes of drug administration. For example, more hepatic and less marrow toxicity might result from oral administration of a drug to a host with a very high rate of conversion of the compound to toxic metabolites in the liver, leaving less drug available to cause toxicity in more distal sites. Differences in immunologic response to bound metabolites or damaged tissues also might alter the clinical features of anticonvulsant-induced disease.

Our patient developed “idiosyncratic” toxicity from two drugs, and we were able to demonstrate an abnormal response to metabolites of both in vitro. Minimal in vitro and no in vivo toxicity were noted from phenobarbital. In one of our previously described patients, drug challenge in vitro showed an abnormality restricted to phenytoin. The response in vitro to phenobarbital and carbamazepine was normal, and the patient tolerated these drugs without toxic sequelae (unpublished observation). Family studies verified the in vitro pattern of responses in both patients. While the number of patients studied to date is small, the data suggest that genetically heterogeneous lesions in drug detoxification may lead to varying patterns of in vivo response to chemically similar compounds. With continued evaluation of the sensitivity and specificity of the in vitro assay used in these studies, it may be possible to screen compounds for safety in patients experiencing adverse drug reactions and further explore the role of electrophilic drug metabolites in human drug toxicity.

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REFERENCES

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