Abnormal Erythroid Maturation in Mice Induced by Isoniazid

By Bernard S. Morse, Dennis Giuliani, Vera Minak, and Murray Nussbaum

Defective erythropoiesis characterizes the sideroblastic anemias. The present studies were undertaken to assess the effects of isoniazid, an inhibitor of heme synthesis, on erythropoietin-induced erythroid maturation in hypertransfused mice. Isoniazid was administered at intervals during a wave of induced erythropoiesis, and the effects were assessed in both the bone marrow and peripheral blood. The data showed that isoniazid interfered with heme synthesis and erythroid maturation. The most inhibitory effect of isoniazid was noted at the intermediate normoblast stage. A transient increase in the reticulocyte nonheme iron pool was also found.

A DIVERSE GROUP OF chemical agents had been implicated in the pathogenesis of acquired sideroblastic anemias. Ethanol, or one of its metabolic products, was reported to interfere with either the phosphorylation of pyridoxine or to accelerate the degradation of phosphorylated pyridoxine. Experimental evidence has indicated that phosphorylated pyridoxine was required for full activity of Δ-ALA synthetase, the first enzyme in the heme biosynthetic pathway. Administration of cycloserine and isoniazid (INH), which inhibit pyridoxal-5-phosphate as well as the phosphorylation of pyridoxine, have resulted in the development of sideroblastic anemia in guinea pigs. Lead, a potent inhibitor of Δ-ALA dehydratase, the second enzyme in the heme biosynthetic pathway, has been amply documented to induce sideroblastic changes in nucleated erythroid cells of both humans and laboratory animals. Using posthypoxic plethoric mice, lead was also found to interfere with erythropoietin (EP)-induced erythropoiesis resulting in a cohort of cells with maturation arrested at the intermediate normoblast stage. The question as to whether the latter defect was specific for lead toxicity or was common to sideroblastic anemias has not been resolved. The present studies were undertaken to assess erythroid maturation in isoniazid (INH)-induced sideroblastic anemia.

MATERIALS AND METHODS

Plethora was induced in 10-wk-old female CF1 mice by the intraperitoneal injection of 1 ml of washed packed isologous red cells on 2 successive days. Erythropoietin (EP) (6 U) was given by tail-vein injection 6 days after the last injection of red cells. Isoniazid (INH), 110 mg/kg, was given by subcutaneous injection in two divided doses at specified intervals after EP (0, 8, 24, 32, 48, 56 hr). This dose and manner of administration of INH was just below the threshold of neurologic toxicity and interfered with heme synthesis for up to 18 hr. Blood samples were collected into versenate by heart puncture from anesthetized animals. Plethoric animals with hematocrits of less than 58% were excluded from the study. Blood samples for red cell $^{59}$Fe...
incorporation were collected 18 hr after the tail-vein injection of plasma-bound $^{59}$Fe. Erythrocytes were then washed three times with saline, lysed with water, and counted in an autogamma scintillation detector. Total red cell $^{59}$Fe incorporation was based on an assumed blood volume of 8% of body weight for hypertransfused mice. After counting was completed, heme was extracted from the hemolysate with 2-butanone as previously described. The heme and nonheme fractions were then recounted for radioiron activity. Tibial marrow cellularity, marrow differential counts, and the stage of nucleated erythroid maturation were assessed. The values presented in the charts and graphs are the mean ± SE (10-20 mice per point).

RESULTS

Untreated plethoric mice displayed nearly complete suppression of erythropoiesis, as evidenced by marrow erythroid precursors of $0.15 \times 10^6$ per tibia, reticulocytes of 0.01%, and an 18-hr RBC $^{59}$Fe incorporation of 0.04%. Forty-eight hours after an injection of 6 U of EP, marrow erythroid activity was maximal ($1.24 \times 10^6$ erythroid precursors per tibia) and declined rapidly thereafter (Table 1). When INH was administered 0 and 8 hr after the EP, marrow erythroid activity and erythroid maturation at 24 hr after EP were comparable to EP-treated controls (Table 1). At 48 hr after EP, however, erythroid activity was approximately 50% of the EP-treated controls which did not receive INH (Table 1). There were approximately half as many late normoblasts as anticipated. Table 1 shows $0.98 \times 10^6$ late normoblasts for EP controls not given INH, compared to $0.47 \times 10^6$ in EP-INH-treated animals. A slight but insignificant reduction in intermediate-stage normoblasts were also noted. Similar findings were noted 48 hr after the EP injection in those mice given INH 24 and 32 hr after the EP (Table 1).

The effects of INH on different maturation stages of EP-induced erythroid differentiation were also assessed using the reticulocyte count and 18-hr RBC $^{59}$Fe incorporation. For these studies, animals were sacrificed 72 hr after EP was administered. When INH was given 48 and 56 hr after EP, a time at which the marrow was composed primarily of late normoblasts (Table 1), there was

![Table 1. Quantitative Bone Marrow Erythroid Response in Hypertransfused Mice Treated With Erythropoietin and Isoniazid](image-url)
Table 2. Effective Erythroid Response in Hypo-transfused Mice Treated with Erythropoietin and Isoniazid

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>18-hr RBC 59Fe Incorporation (%)</th>
<th>39Fe Fes (%)</th>
<th>Reticulocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP</td>
<td>72</td>
<td>9.86 ± 1.11</td>
<td>78.3 ± 1.3</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>EP-INH (0, 8)†</td>
<td>72</td>
<td>5.25 ± 0.54</td>
<td>78.3 ± 0.7</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>EP-INH (24, 32)</td>
<td>72</td>
<td>3.14 ± 0.43</td>
<td>77.2 ± 0.5</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>EP-INH (48, 56)</td>
<td>72</td>
<td>9.22 ± 0.98</td>
<td>57.4 ± 1.9</td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.04 ± 0.01</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

Animals injected 6 days after last red cell transfusion. Values represent mean ± SE, 8–18 animals per point.

† Time of sacrifice in hours after erythropoietin injection.
† Per cent of red cell 59Fe activity extracted with 2-butanone.
† Refers to interval in hours between erythropoietin (EP) and isoniazid (INH), e.g., EP-INH (24, 32) indicates that INH was given 24 and 32 hr after EP.

little discernible effect on the subsequent release of these cells into the peripheral blood as reticulocytes (Table 2, Fig. 1). Red cell 59Fe incorporation did not differ significantly from EP-treated controls; however, the portion of RBC 59Fe incorporation utilized for heme synthesis was significantly reduced in the INH-treated animals. An increase in the red cell nonheme iron pool was found (Table 2). A comparable reduction of the reticulocyte response to EP was observed when INH was given at either 0 and 8 hr or 24 and 32 hr after EP (Table 2, Fig. 1). Values for RBC 59Fe incorporation were also decreased at these intervals compared to EP-treated controls. The lowest values for RBC 59Fe incorporation were recorded when INH was given at 24 and 32 hr after EP (Table 2, Fig. 1). The red cell nonheme iron pool in this cohort of cells after release into the peripheral blood was not increased (Table 2).

**DISCUSSION**

Isoniazid has been implicated as an etiologic factor in the pathogenesis of the acquired sideroblastic anemias when administered with other antitubercu-

Fig. 1. The 72-hr effective erythroid response of control plethoric mice to 6 U of erythropoietin (EP) administered on day 0 is represented by the shaded area. Hours on abscissa indicate interval between injection of EP and INH. Values are represented as per cent of EP-treated controls. The solid line ———— indicates 18-hr RBC 59Fe incorporation, and the interrupted line indicates the reticulocyte response. Vertical lines through the points indicate ±1 SEM.
lous medications, i.e., cycloserine, pyrazinamide, etc.11,13,14 Clearly, the present data indicate that INH alone interferes with heme synthesis and erythroid maturation in the mouse. When INH is administered either at 0 and 8 hr or 24 and 32 hr after EP, both marrow erythropoiesis and effective erythropoiesis are diminished to the same extent. It is suggested that cells differentiating in response to EP undergo an arrest in the maturation sequence in the presence of INH. It would appear that the arrest occurs during the maturation of intermediate normoblasts. This contention is supported by the observation that, in the presence of INH, half as many late normoblasts are formed (Table 1).

A greater erythro-inhibitory effect of INH would have been found as the interval between EP and INH injections was decreased if the arrest occurred prior to the formation of intermediate normoblasts. If the arrest occurred during the late normoblast stage, then a substantial erythro-inhibitory effect would be expected when INH was given at 48 and 56 hr after EP. Little if any effect was, in fact, observed, indicating orderly maturation and release of these cells into the peripheral blood as reticulocytes (Table 2). Values for iron incorporation, on the other hand, revealed substantial differences for the EP-INH-treated animals compared to EP-treated controls. An increased intracellular nonheme iron pool characterized the newly formed red cells when INH was given at 48 and 56 hr after EP. Despite the presence of this increased nonheme iron pool, the cells, for the most part, gained access to the peripheral blood. When INH was given at the earlier intervals, i.e., 0 and 8 or 24 and 32 hr after EP, the cells released into the peripheral blood between 54 and 72 hr did not contain an increased nonheme iron pool. Possible explanations for this finding include utilization of nonheme iron for heme synthesis,15 loss by rophocytosis,16 or in situ cell death.

The disturbance of erythroid maturation and the development of sideroblastic anemia described herein is somewhat similar to that of lead toxicity.11 A similar experimental design was used, and the disturbance of erythroid maturation is best explained by a selective toxic effect of INH on intermediate-stage normoblast maturation. As with lead, the effects of INH on late normoblasts are minimal. These agents differ, however, with respect to their effect on the red cell nonheme iron pool, consistently increased after lead and transiently increased after INH (Table 2). This observation tends to favor the suggestion that the increased red cell nonheme iron pool induced by INH is utilized for heme synthesis as the inhibitory effects of INH are reduced by metabolism, excretion, etc. Similar findings have been reported by Ponka and Neuwirt following incubation of rabbit reticulocytes in vitro with INH and $^{59}$Fe.15

Recently, White, Brain, and Ali17 reported that globin synthesis was defective in patients with sideroblastic anemias. Deficiency of $\alpha$-globin chain synthesis and free $\alpha$- $\beta$-chain dimers were reversible in vitro by the addition of heme. They concluded that the underlying defect in sideroblastic anemia was heme deficiency. In the present studies, INH, presumably by interfering with pyridoxal-5-phosphate, decreased the synthesis of heme, with an increased portion of iron entering the intracellular nonheme iron pool. Heme deficiency and defective globin synthesis as well as nonheme iron accumulation all appear to be required
for the full development of sideroblastic anemia. It is suggested that, in the case of INH-induced sideroblastic anemia, this latter phenomenon is transient.

ACKNOWLEDGMENT

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REFERENCES

12. Morse BS: Unpublished observations
Abnormal erythroid maturation in mice induced by isoniazid

BS Morse, D Guiliani, V Minak and M Nussbaum