Tin-Protoporphyrin Suppression of Hyperbilirubinemia in Mutant Mice With Severe Hemolytic Anemia

By Shigeru Sassa, George S. Drummond, Seldon E. Bernstein, and Attallah Kappas

Tin-protoporphyrin is a potent competitive inhibitor of heme oxygenase both in vivo in animals and in vitro in isolated enzyme preparations, and when administered to neonatal rats, prevents the development of postnatal hyperbilirubinemia. In this study we examined the effect of the metalloporphyrin on the activity of heme oxygenase in liver, kidney, and spleen, and on the level of bilirubin in plasma in three types of anemic mutant mice with severe hereditary anemias in mice. Homozygotes of all three types have a severe intramedullary and intravascular hemolysis, marked jaundice, gallstones, hepatosplenomegaly, cardiac hypertrophy, bone marrow hyperplasia, and reticulocytosis (60%-90%). Affected homozygotes for these gene mutations display severe hemolysis with a red cell half-life of less than 2 days compared with a normal value of approximately 50 days. The rate of erythrocyte destruction in these mice exceeds that in human newborns with Rh incompatibility or adults with acute hemolytic crises in such disorders as sickle cell disease or glucose-6-phosphate dehydrogenase deficiency. Mice were maintained on Purina Rodent Lab Chow diet Formula 5001 in the Laboratory Animal Research Center of Rockefeller University. They were fed and watered ad libitum. The principles of laboratory animal care as promulgated by the National Society of Medical Research were observed in both institutions. Tin-protoporphyrin was purchased from Porphyrin Products, Logan, UT, and other chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

Mice were injected at subcutaneous sites with tin-protoporphyrin in various doses as described below and in the figure legends. The tin-protoporphyrin solution was prepared as follows: 35.75 mg of tin-protoporphyrin was first dissolved in 1 ml of 0.5 N NaOH. Three milliliters of 0.9% NaCl was added with constant stirring. The pH of the solution was then carefully brought down to 7.5 with 0.5 N HCl. Then, 0.9% NaCl was added to a final volume of 5 ml. Control animals received equivalent amounts of saline. Blood was obtained in heparinized, hematocrit capillary tubes by puncture of the retro-

Materials and Methods

The animals used in this study included mutants 8-12 mo of age afflicted with hemolytic anemia (genotype ha/ha), normoblastosis (genotype nh/nh), or spherocytic anemia (sph/sph). All genotypes were prepared at the Jackson Laboratory on the same genetic background (WBB6F1). These mutant genes behave as autosomal recessives and are not allelic with those causing other hemolytic diseases. We report that the administration of tin-protoporphyrin to anemic mutants homozygous for severe hemolytic disease results in substantial inhibition of heme oxidation in liver, spleen, and kidney and in significant reduction of plasma bilirubin levels. Tin-protoporphyrin thus has the capacity to significantly inhibit in vivo hemodegradation and to concurrently diminish plasma bilirubin levels in severe chronic hemolytic disorders.

Recent studies from this laboratory have shown that a marked inhibition of tissue heme oxidation rates in vivo and a suppression of excessive plasma bilirubin levels in the neonatal animal can be produced by the use of a synthetic metalloporphyrin that acts as a competitive inhibitor of the heme oxygenase reaction in such organs as liver, spleen, and kidney. Tin-protoporphyrin is an especially effective competitive inhibitor of this reaction, and when administered in single small doses to newborn rats at birth, it can entirely prevent the postnatal hyperbilirubinemia that occurs in these animals. Its remarkable potency as a competitive inhibitor of the heme oxygenase reaction is reflected in the fact that the K0 of tin-protoporphyrin is extremely low (0.01-0.03 μm) for the microsomal enzyme as well as for the homogeneously purified enzyme. Among other synthetic metalloporphyrins that inhibit heme oxygenase, only chromium-protoporphyrin has to date been shown to have a comparable biologic action to that of tin-protoporphyrin in suppressing hyperbilirubinemia in the neonatal animal.

In order to further explore the potential of tin-protoporphyrin for suppression of excessive plasma bilirubin levels by inhibiting heme oxidation in vivo, we have studied the effect of the metalloporphyrin on the jaundice that occurs in anemic mutant mice with profound hemolytic disease.

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orlinal sinus. Approximately 150 μl was obtained at each sampling. Animals were sacrificed only for the determinations for heme oxygenase activity in various tissues; sacrifice was not necessary for the time-course studies of plasma bilirubin levels. All assays used in this study have been described previously.1,15

RESULTS AND DISCUSSION

Effect of Single Doses of Tin-protoporphyrin on Plasma Bilirubin Levels in sph/sph and nb/nb Mice

Plasma bilirubin levels declined promptly (within 24 hr) after single injections of the metalloporphyrin in a dose-dependent manner; data for sph/sph mice are shown in Fig. 1A. At a dose of 10 μmole/kg, the plasma bilirubins remained low (~35%) for 3 days before returning to control levels by day 5. At a dose of 100 μmole/kg, the plasma bilirubin remained below normal (~35%) for the 5-day period of study. An intermediate response was produced by a dose of 50 μmole/kg (data not shown).

The effects of two doses (100 μmole/kg) of tin-protoporphyrin administered 1 wk apart on plasma bilirubin levels in mice with hemolytic anemia were studied over an 8-wk period; the results for nb/nb mice, shown in Fig. 1B, are comparable to those for ha/ha and sph/sph mice. Plasma bilirubin concentrations dropped substantially after the first injection of the metalloporphyrin and remained ~40% below pretreatment levels for the 8-wk period of study. In none of the treated animals did the plasma bilirubin revert entirely to normal.

Effects of Repeated Doses of Tin-protoporphyrin on Plasma Bilirubin Levels and Tissue Heme Oxygenase Activities in ha/ha Mice

In order to determine whether intensive treatment with tin-protoporphyrin was capable of suppressing the elevated plasma bilirubin in these severely hemolytic mice to normal levels, the metalloporphyrin (100 μmole/kg) was administered once weekly over a 16-wk period to ha/ha mice; selected animals were sacrificed at 2 wk in order to determine heme oxygenase activities in liver, kidney, and spleen.

With repeated weekly injections of tin-protoporphyrin, plasma bilirubin levels in these mice dropped promptly (within 1 day) and were maintained at steady levels 50% below those in untreated animals for the entire 16-wk period of study. After the initial decline, plasma bilirubin stabilized at levels above normal, but approximately one-half those of the pretreatment period (Fig. 1C). Tissue heme oxygenase activities in treated animals were markedly diminished by tin-protoporphyrin treatment, hepatic enzyme activity being depressed to 75% below controls, and in spleen and kidney, to 70% and 80% below controls, respectively (Fig. 2).

These studies indicate that tin-protoporphyrin can significantly lower, for long periods of time, the elevated plasma bilirubin in three genetically distinct types of severe hemolytic anemia in mutant mice. Evidence of a concurrent and pronounced inhibition of tissue heme oxidation rates suggests that the ability of the metalloporphyrin to lower plasma bilirubin levels derives in substantive degree from its capacity to act as an inhibitor of heme oxygenase and thus to diminish production of bile pigment.

The marked affinity of the metalloporphyrin for the catalytic site of heme oxygenase,1,4 and the fact that tin-protoporphyrin is not oxidatively degraded to bile pigment by the enzyme,1,4 may account for the prolonged biologic effects observed in vivo. Despite inten-
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Fig. 2. Effect of tin-protoporphyrin on the activity of heme oxygenase. Tin-protoporphyrin (100 μmole/kg body weight) was injected subcutaneously to ha/ha mice on days 0 and 7, then mice were sacrificed on day 14. Heme oxygenase activity was determined as described previously. Data are the means of 2 determinations.

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REFERENCES
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