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Prevention of Neonatal Hyperbilirubinemia in Rhesus Monkeys by Tin-protoporphyrin

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Summary

Rhesus monkey infants were injected subcutaneously at birth with 12 to 100 μmol of tin-protoporphyrin IX, a competitive inhibitor of microsomal heme oxygenase. The elevated unconjugated serum bilirubin levels of the neonates receiving this metalloporphyrin rapidly declined to near adult levels by 24–30 h. Control neonates which received an injection of saline exhibited normal physiologic hyperbilirubinemias of from 3–6 mg/dl by 12–24 h as expected. These studies establish the effectiveness of tin-protoporphyrin IX in depressing bilirubin production and preventing physiologic hyperbilirubinemia in simian neonates. Two of six animals receiving the metalloporphyrin exhibited signs of toxicity.

Physiologic jaundice in the newborn human infant has been the subject of intensive investigation for many years (10, 13). Studies have been generally limited to the measurement of single physiologic mechanisms in neonates; this has made interpretations difficult as to the effects of bilirubin overload (10), deficient hepatic UDP-glucuronyltransferase activity (4, 7), and defective hepatocellular uptake of unconjugated bilirubin (8, 14). Due to the absence of physiologic jaundice in the guinea pig (8) and the presence of only a transient hyperbilirubinemia (<1 mg/dl) in neonatal rats (6), the newborn rhesus monkey which exhibits a marked physiologic hyperbilirubinemia appears to be the animal model of choice for future studies (10).

Rhesus monkey neonates exhibit a two-phase pattern of physiologic unconjugated hyperbilirubinemia similar to that observed in humans, although of shorter duration (10). It has also been observed that newborn rhesus monkeys excrete bilirubin conjugates into bile at rates 5-fold greater than those of mature animals (9, 10). The increased load of bilirubin presented to the human neonate's liver could possibly occur from increased bilirubin synthesis (16) and/or augmented enteric reabsorption of bilirubin (18). Interruption of the enterohepatic circulation of bilirubin through bile diversion from the intestine significantly reduces the bilirubin load presented to the liver (18). It has been postulated that the bilirubin monoglucuronide excreted by the neonate into the intestine (2, 3) may be more easily deconjugated and reabsorbed into the enterohepatic circulation than is bilirubin

diglucuronide which is the primary pigment excreted by adult primates (11).

Since bilirubin synthesis in human neonates is at least two to three times greater on the first day of life than in adults (16), any biologic process which slows synthesis in neonates should in turn reduce the bilirubin load and ameliorate physiologic neonatal hyperbilirubinemia. Maines (15) and Drummond and Kappas (6) recently studied the effects of various metalloporphyrins in rats on the activity of hepatic, splenic, and renal microsomal heme oxygenase, a rate-limiting enzyme in bilirubin production. Tin-protoporphyrin IX was particularly effective as a competitive inhibitor of heme oxygenase. The substantial elevations of tissue heme oxygenase activities that are present immediately after birth in neonatal rats were prevented by a single administration of tin-protoporphyrin (5, 6). Serum bilirubin levels declined within 24 h and remained near adult levels thereafter.

The present study was designed to simply test the effects of tin-protoporphyrin IX on simian neonates, which like human infants, exhibit a similar physiologic hyperbilirubinemia.

MATERIALS AND METHODS

Twelve rhesus monkey (*Macaca mulatta*) neonates were obtained for study at the California Primate Research Center. All pregnancies were uncomplicated and delivered by either cesarian section at 158–162 days of gestation as determined by timemated breedings or by normal spontaneous birth. All infants were successfully nursed by their mothers except for animals C 2 and T 25 which received Enfamil (Mead Johnson) by bottle. Chronologic age and body weight at the time of first bleeding, mode of delivery, and subcutaneous injected doses of tin-protoporphyrin IX or saline are presented in Table 1. Untreated neonates served as controls by receiving a total of 2 ml of sterile physiologic saline injected subcutaneously at birth in 2 divided doses. Five other neonates received between 12 and 100 μmol of tin-protoporphyrin IX (Porphyrin Products, Logan, UT) subcutaneously, also in 2 divided doses. The tin-protoporphyrin IX was first dissolved in 0.2 ml of 6 N NaOH and 0.5 ml of NaH_2PO_4 buffer (pH 7.4). After thorough mixing, 1 ml of sterile saline was added and the solution was carefully back-titrated dropwise with 6 N HCl to near neutrality to prevent precipitation of the tin-protoporphyrin. Final volume injected was 2 ml. Serum samples were obtained at birth or shortly thereafter and in most cases up to 96 h. Approximately 1 ml of venous blood was drawn at each bleeding; the total amount of blood drawn in the neonates during the 96-h period never exceeded 15% of blood volume.

Serum bilirubin concentrations were determined by the method of Jendrassik and Grof (12). Serum unconjugated bilirubin concentrations were estimated from the differences between the total and direct-reacting pigment and accounted for most of the pigment measured. Since it has been previously established that increases in the total serum bilirubin levels in normal neonates are primarily due to elevations in unconjugated pigment as confirmed by high pressure liquid chromatography, it appeared appropriate to present bilirubin levels as the total pigment in serum in Figures 1 and 2. Since a mild conjugated hyperbilirubinemia was observed in two neonates at 60 h after tin-protoporphyrin injection, serum bilirubin values were only presented in monkeys T 25a and T 100b in Figure 2 prior to this time.

Table 1. *Chronological age and body weight at time of first bleeding and the injection; mode of delivery; sex; and treatment with tin-protoporphyrin IX (Tin-P)*

Animal*	Age (hr)	Body weight (*g*)	Delivery†	Sex	Treatment
C 1	1	439	CS	F	2 ml saline
C 2	1	635	CS	M	2 ml saline
C 3	1	480	SV	M	2 ml saline
C 4	1	565	SV	F	None
C 5	1	460	SV	F	None
C 6	6‡	460	SV	M	None
T 12	1		CS	M	12 μmol Tin-P
T 25a	1	590	CS	M	25 μmol Tin-P
T 25b	1	545	CS	M	25 μmol Tin-P
T 50	1	650	CS	M	50 μmol Tin-P
T 100a	1	600	SV	M	100 μmol Tin-P
T 100b	6‡	540	SV	M	100 μmol Tin-P

* C, control; T, tin-protoporphyrin treated.

† CS, cesarian section delivery; SV, spontaneous vaginal delivery.

‡ Estimated at 6 ± 2 h.

RESULTS

Neonatal physiologic unconjugated hyperbilirubinemia in six control rhesus neonates receiving 2 ml of saline subcutaneously

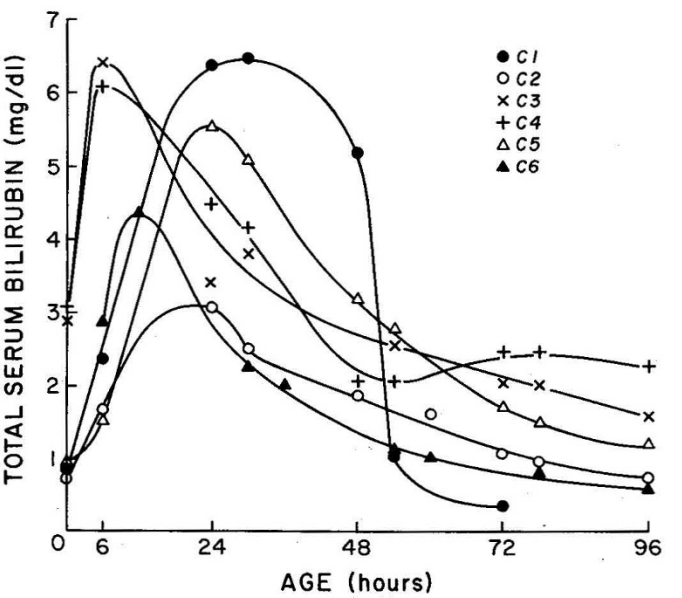

Fig. 1. Total serum bilirubin concentrations (physiologic hyperbilirubinemia) in six control rhesus monkey neonates.

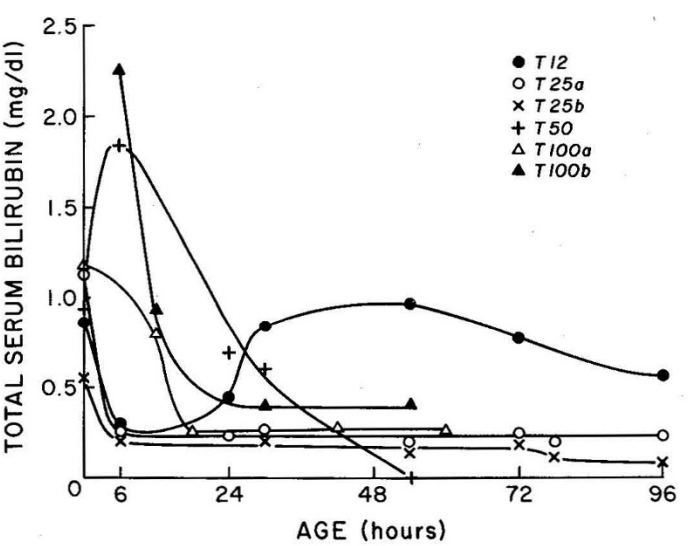

Fig. 2. Total serum bilirubin levels in rhesus monkey neonates in response to the subcutaneous injection of various doses of tin-protoporphyrin. All neonates were injected at the time of birth except for T 100b, which received the metalloporphyrin at approximately 6 h after birth. *Numbers* assigned to neonates indicate the dose injected in micromoles.

is presented in Figure 1. Unconjugated hyperbilirubinemia in neonates following various subcutaneous doses of tin-protoporphyrin at birth can be observed in Figure 2. Please note the different scales of bilirubin concentrations in Figures 1 and 2. Control neonates in all cases exhibited the predicted physiologic hyperbilirubinemia as previously reported by Gartner *et al.* (10), whereas serum bilirubin levels in all but one infant receiving the tin-protoporphyrin rapidly declined to levels less than 0.4 mg/dl by 24–30 h. The serum bilirubin concentration in the neonate (T 12) which received the lowest dose (12 μmol) of tin-protoporphyrin initially declined rapidly to 0.25 mg/dl by 6 h and unexplainably rebounded to 0.95 mg/dl at 54 h postinjection.

Two neonates (T 25a, T 100b) that received 25 and 100 μmol of tin-protoporphyrin, respectively, exhibited slightly elevated serum direct reacting bilirubin levels of 0.5–0.7 mg/dl at 60 h postinjection. These animals subsequently developed differing signs of toxicity following injection. Neonate T 25a developed a dermatitis after 4 days. Biopsies revealed multiple cutaneous bullae with an intact epidermis and a mild lymphohistiocytic perivascular dermal inflammation. The lesions healed within 3 weeks without scarring and a rechallenge with tin-protoporphyrin at 8 weeks was uneventful. Neonate T 100b died 7 days after injection. Gross observations at necropsy revealed diffuse petechial hemorrhages and edema, hemorrhage, and localized necrosis of the extremities. Gross and histologic examination of liver, spleen, and kidneys showed evidence of infarction; in addition, there was histologic evidence of disseminated arterial thrombosis.

DISCUSSION

The substantial elevations in heme oxygenase activities in the liver, spleen, and kidneys of rats that occur following birth have been shown to be prevented by a single dose (10 μmol/kg) of tin-protoporphyrin (5). *In vitro* studies on microsomal heme oxygenase indicate that this metalloporyphyrin is also a potent competitive inhibitor of the oxidation of heme to bilirubin in human spleen (5). These studies suggest that the lowest single effective dose of tin-protoporphyrin IX to prevent postnatal hyperbilirubinemia in rats was 10 μmol/kg; doses ranging from 5 to 100 μmol/kg were tested and apparently free from toxicity (5).

Rhesus neonates receiving tin-protoporphyrin also quickly responded by exhibiting decreasing serum levels of unconjugated bilirubin and indicated its effectiveness in inhibiting heme oxygenase activity and bilirubin production in primate species. Since tin-protoporphyrin was administered in the range of 12–100 μmol in a single dose to the rhesus neonates, the unexplained toxicity in 2 neonates after 54 h may have been due to either a drug sensitivity or the high level administered in a single dose. Lower levels of drug administration may well prove to be free of toxicity. These observations deserve further study since the multiple administration of amounts totaling 500 μmol/kg of tin-protoporphyrin to neonatal rats has been reported to produce no apparent acute or chronic toxicity (5, 6).

Whether this transient suppression of heme oxidation by tin-protoporphyrin IX in neonates could lead to heme accumulation in the serum and tissues and subsequent toxicity is not clear. It is known, however, that daily doses of 800 mg of heme administered to adult patients with hepatic or erythroid porphyria produce no observable toxicity (1). Since heme is a normal constituent of bile and readily appears in bile after its infusion (17), it may not accumulate significantly in tissues following heme oxygenase inhibition. It is apparent from this brief investigation that the use of tin-protoporphyrin IX may prove to be useful in the prevention of neonatal jaundice and deserves further study.

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