AN ALTERNATE PATHWAY FOR BILIRUBIN CATABOLISM

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1. Introduction

Parturition associated hyperbaric oxygen toxicity is manifest in newborn humans as increased heme catabolism leading to hyperbilirubinaemia. Functionally mature hepatocytes are not available to deal with the extra load of bilirubin and physiological jaundice ensues with increased levels of circulatory unconjugated bilirubin. The mature hepatocyte in contrast is equipped with UDP-glucuronyl transferase and can render the bile pigment hydrophilic by conjugation with glucuronic acid [1]. Pathways for bilirubin degradation involving photooxygenation are mediated by singlet oxygen [2].

The first-order rate constant for bilirubin degradation in an in vitro system can be stimulated by an order of 10 by incorporation of a superoxide generating system such as xanthine–xanthine oxidase. The stimulation caused by superoxide is neutralized by the inclusion of superoxide dismutase in the assay mixture [3,4]. Does the superoxide radical function as a mediator of bilirubin degradation in vivo? Here we show that superoxide-mediated bilirubin degradation is an alternate catabolic pathway for bilirubin metabolism.

2. Materials and methods

Adult albino rats (Charls Foster Strain) 150–200 g from Central Drug Res. Inst. Colony maintained on standard animal house diet (Hindustan Lever pellet diet) were employed for in vivo experiments.

Protein malnourished rats were fed a 6% casein diet for 3 weeks while control groups were fed an 18% casein diet for 3 weeks [5].

2.1. *Administration of bilirubin*

Bilirubin was dissolved in a few drops of 0.1 N NaOH and volume made up with 0.1 M phosphate buffer (pH 7.4) and i.p. injected at 50 mg/kg body wt immediately. The control group received an equal amount of 0.1 M phosphate buffer (pH 7.4).

2.2. *Administration of xanthine and bilirubin*

Xanthine was dissolved in 0.1 N NaOH, heated and adjusted to pH 8.4 with 0.1 M phospate buffer and i.p. administered at 2.5 mg/kg body wt while rats receiving 0.1 M phosphate buffer (pH 8.4) served as control. At 60 min after administration of xanthine or buffer, bilirubin was i.p. injected at 50 mg/kg body wt.

2.3. *Collection of blood and estimation of bilirubin*

Blood was withdrawn through the occular vein in heparinised capillary tubes every 30 min. Bilirubin in the plasma was estimated by a modified technique [6] using crystalline bilirubin as a standard.

2.4. *Chemicals*

Bilirubin was procured from Merck, Darmstad. Xanthine was obtained from Sigma Chem. Co. OH. All other reagents used were of analytical grade.

3. Results and discussion

Rats administered xanthine (i.p.) prior to bilirubin showed a plasma bilirubin half-life of 100 min as compared to control group showing a half-life of 180 min (fig.1). The saturation of xanthine–xanthine oxidase in vivo, generates a greater amount of superoxide which in turn catalyses a faster rate of degradation of

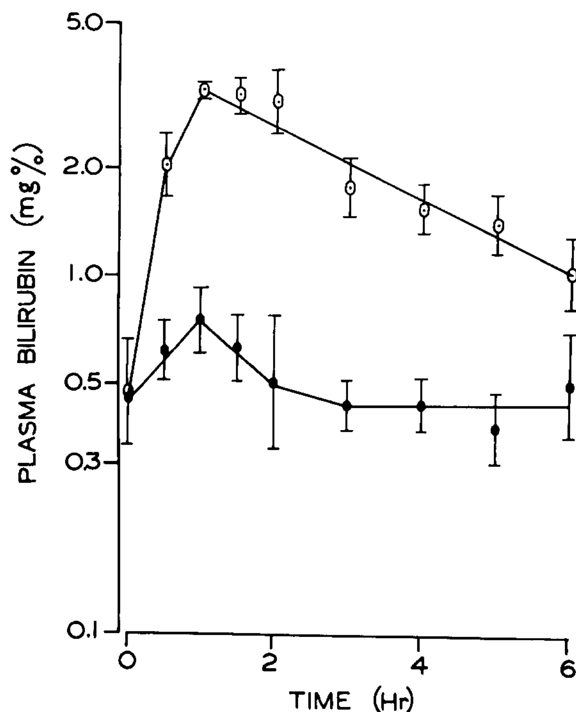

Fig.1. Effect of xanthine administration on bilirubin degradation in experimental hyperbilirubinemia. Animals injected phosphate buffer 60 min before administration of 50 mg bilirubin solution/kg body wt (○——○). Animals i.p. injected 2.5 mg/kg body wt xanthine intraperitoneally, 60 min before the administration of 50 mg/kg body wt (●——●). Each bar represents arithmetic mean of observations from 6 animals and vertical bars represent standard deviation.

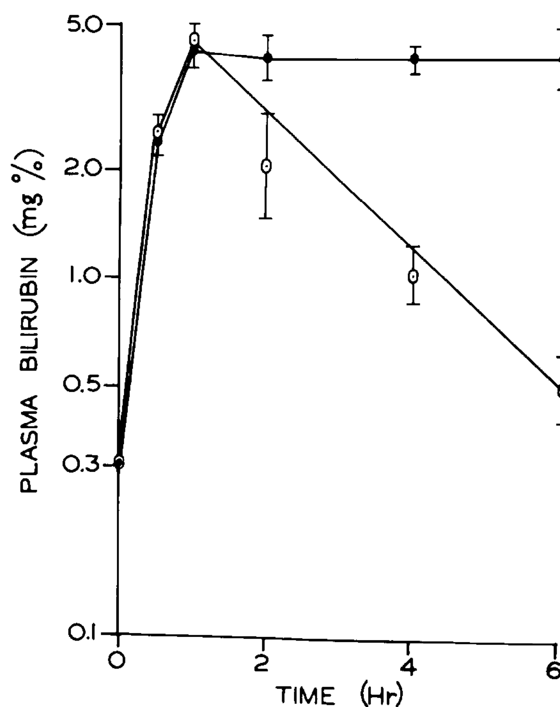

Fig.2. Inhibition of bilirubin metabolism clearance rate on protein malnutrition in rats. Normal animals administered 50 mg bilirubin solution/kg body wt (○——○). Protein malnourished rats administered 50 mg bilirubin solution/kg body wt (●——●). Each bar represents arithmetic mean of observations from 6 animals and vertical bars represent standard deviation.

bilirubin. Generation of superoxide radical in vivo could thus stimulate the bilirubin plasma clearance rate in experimental hyperbilirubinemic animals.

Further support for this emerged from the observation that plasma half-life of bilirubin in protein malnourished rats was much higher than in control rats (fig.2). Xanthine oxidase levels are regulated by dietary proteins [7] and very low levels have been reported in protein malnourished rats [8]. During protein malnutrition the glucuronide conjugation is not affected, since the drug paracetamol known to be excreted through conjugation shows a similar plasma half-life in both control as well as malnourished rats [9]. Thus the enhanced bilirubin half-life in malnourished rats may be a sequel to lowered xanthine oxidase. Superoxide generated in situ by xanthine–xanthine oxidase or other radical oxygen generating systems such as cytochrome P448 [10] would initiate a chain reaction leading to bilirubin degradation and the appearance of hydrophilic metabolites in jaundiced human neonates [11], Gunn rats [12] with congenital UDPGT deficiency and photooxygenation products of bilirubin.

It is tempting to speculate that hyperbilirubinemia is a natural defence mechanism for encountering hyperbaric stress-associated oxygen toxicity, where bilirubin functions in the sequestation of superoxide radical.

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