Urinary excretion of biopyrrin in unconjugated hyperbilirubinemia

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Summary We determined the urinary excretion of biopyrrin, an oxidative metabolite of bilirubin, in three jaundiced subjects with unconjugated hyperbilirubinemia and in eight normal controls. We used an enzyme-linked immunosorbent assay (ELISA) and an anti-bilirubin monoclonal antibody, 24G7 (Shino-Test Corporation, Kanagawa, Japan). The biopyrrin excretions, expressed as the ratio in urine of biopyrrin to creatinine, were measured in random urine specimens from jaundiced adults and were compared to the same in random urine specimens from normal adults. The central 95% of the distribution range, defined by us as the reference range for the urinary biopyrrin/creatinine excretion, was 0.5 - 3.3 μmol/g in presumably healthy adults. The biopyrrin/creatinine excretions from the jaundiced adults were significantly higher than the reference range (p<0.05). We found that for the 24-hr biopyrrin excretion from normal subjects, and that the central 95% of the data was 0.9 - 1.5 μmol/day; the amounts in the jaundiced adults were significantly higher than the reference range (p<0.05). Our observations suggest that there is an enhanced bilirubin catabolism to biopyrrin in subjects with unconjugated hyperbilirubinemia.

Key words: Bilirubin, Jaundice, Gilbert syndrome, Random and 24-hr urine, Biopyrrin, Hyperbilirubinemia

1. Introduction

Biopyrrin is an oxidative metabolite of bilirubin¹. Biopyrrin is a tripyrro moiety of bilirubin, including two regioisomers (biotripyrрин-a, 1,14,15,17-tetrahydro-2,7,13-trimethyl-1,14-deoxy-3-vinyl-16H-tripyrrin-8,12-dipropionic acid and biotripyrrin-b, 1,14,15,17-tetrahydro-3,7,13-trimethyl-1,14-deoxy-2-vinyl-16H-tripyrrin-8,12-dipropionic acid: Fig. 1). For many years, bilirubin was regarded as a potentially toxic waste product formed during heme catabolism. However, recent evidence suggests that bilirubin is a physiological antioxidant that may scavenge oxygen and peroxyl radicals as efficiently as a-tocopherol². Circulating bilirubin functions as a suicide antioxidant; it is catabolized to biopyrrin and is excreted in urine. We now have an assay method for biopyrrin in urine that uses an enzyme-linked immunosorbent assay (ELISA) and an anti-bilirubin monoclonal antibody, 24G7³. The antibody has an epitope in the dipyrrole...
region of bilirubin, and it reacts with biopyrrin. Because biopyrrin is formed from bilirubin, it was of interest to us to learn whether urinary excretion of biopyrrin is increased in jaundiced subject. To this end, we evaluated the urinary excretion of biopyrrin in three subjects with unconjugated hyperbilirubinemia.

2. Materials and Methods

1. Subjects

For two successive days, we collected random urine specimens from the three jaundiced subjects. Two were women aged 31 y (W1) and 60 y (W2) and one was a man aged 41 y (M1), all of whom were presumed to be in good health except for the unconjugated hyperbilirubinemia. Their serum concentrations of unconjugated bilirubin were 19, 24, and 16 mg/L, respectively. Serum conjugated bilirubin was not detected using an HPCL method[6]. Random urine specimens were also collected from eight presumably healthy, non-jaundiced adults (three men, aged 26, 37 and 53 y and five women, aged, 22, 30, 33, 34 and 39 y). Written informed consent was obtained from all subjects, and our study was in compliance with our institution's rules for human experimentation.

2. Methods

We measured concentrations of biopyrrin in urine by ELISA using an anti-bilirubin monoclonal antibody, 24G7 (Shino-Test Corporation, Kanagawa, Japan)[7]. We standardized the ELISA method with unconjugated bilirubin, and expressed the concentration of biopyrrin in terms of the equivalent unconjugated bilirubin concentration. For example, 1.0 µmol/L biopyrrin is equivalent to 1.0 µmol/L unconjugated bilirubin. The ELISA test was linear from 0.1 to 3.2 µmol/L. A within-day assay for five consecutive days yielded a mean ± SD of 0.66 ± 0.039 µmol/L. The between-day assay for those five days showed a mean ± SD of 0.57 ± 0.040 µmol/L. We also measured the creatinine in urine, and expressed the biopyrrin excretion in random urines as the ratio of the biopyrrin to the creatinine concentrations.

3. Analysis of data

We tested for statistically significant differences using the Mann-Whitney U test for non-Gaussian variables. We defined a statistically significant difference or agreement to be present if p<0.05.

3. Results

Our non-jaundiced subjects collected 4 to 12 random urine specimens (8 times on average) in 24
hours. A total of 240 random urine specimens were obtained. The biopyrrin/creatinine ratio in the random specimens ranged from 0.2 to 3.2 $\mu$mol/g (median, 1.31 $\mu$mol/g and mean, 1.39 $\mu$mol/g). Significant gender differences were not found. The frequency distributions of biopyrrin excretion in the random urine were non-Gaussian (Fig. 2). Because the distribution was log-normal, and therefore the use of a geometric mean appeared better suited for this study, we calculated the central 95% distribution range, i.e., the reference range being defined by: antilog of [log mean ± (2 log SD)]. We obtained a reference range of 0.5 - 3.3 $\mu$mol/g for biopyrrin/creatinine excretion in random urine specimens.

We also investigated the urinary excretion of biopyrrin in the three subjects with unconjugated hyperbilirubinemia. Biopyrrin excretion in each of the random urine specimens varied with the time of day; we found no trends or correlations (Fig. 3). We made the same observation in the reference population. When biopyrrin/creatinine excretion in random urine specimens from jaundiced subjects were compared to those from non-jaundiced subjects, the excretion ratios from random urine specimens obtained from W1 were all above the upper reference limit (p<0.001). An increased excretion was also found once in W2 and twice in M1 during two successive days.

The total amounts of biopyrrin excreted in a 24-hr urine were calculated for our subjects as follows: On first morning we started the collections, the first urine was discarded. We then collected all urine voided for the next 24 hours, including the first from the next morning. We calculated the amounts of biopyrrin excreted in urine in 24 hours from the sum of the biopyrrin obtained in the random urine specimens described above. The total amounts of biopyrrin excreted in 24-hr urine from the jaundiced subjects were significantly higher than those from the normal subjects (p<0.05). The total amounts excreted were 6.6 and 7.1 $\mu$mol/day from W1; 1.5 and 2.2 $\mu$mol/day from W2; and 5.4 and 5.5 $\mu$mol/day from M1. Each pair (e.g., 6.6 and 7.1) lists data from day 1 and day 2, respectively. In normal subjects, the 95% distribution range for total biopyrrin excretion was 0.9 - 1.5 $\mu$ mol/day.

4. Discussion

The urinary excretion of biopyrrin in conjugated hyperbilirubinemia could not be measured using the anti-bilirubin monoclonal antibody, 24G7, since that antibody reacted with both biopyrrin and conjugated bilirubin$^5$. Therefore, we investigated the urinary excretion of biopyrrin in unconjugated hyperbilirubinemia. The latter type of hyperbilirubinemia exclusively observed in some of neonates, hemolytic diseases, and in congenital nonhemolytic jaundice (i.e., Gilbert and Crigler-Najjar syndromes) showed elevated serum unconjugated bilirubin. Those subjects, however usually showed little or no detectable conjugated bilirubin in serum or urine$^6$, due to the lowered activity or deficiency of the conjugating liver enzyme, bilirubin UDP-glucuronosyltransferase$^{5,6}$. Our three subjects would be considered mild cases of Gilbert syndrome. In healthy adults having normal bilirubin glucuronidation, most or all the bilirubin is rapidly

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excreted into bile, with little or no conjugated bilirubin observed in urine.

In this study, we found that the urinary excretion of biopyrrin in unconjugated hyperbilirubinemia was significantly higher than that in normal subjects. If we artificially measured conjugated bilirubin as biopyrrin, the excretion would be lower in our jaundiced subjects compared to normal ones. To our knowledge, this is the first report on biopyrrin excretions in jaundiced subjects. In another study, urinary excretion of biopyrrin was reported to be elevated in several diseases: sepsis, ischemic heart disease\(^7\), congestive heart failure\(^8\), atopic dermatitis, surgical stress\(^9\)\(^10\) and psychological stress\(^11\)\(^12\), wherein oxidative injury or "stress" was present. Our three cases were largely in good health except for slight jaundice. In addition, they suffered from no psychological stress during this study. We can conclude that there was an enhanced bilirubin catabolism to biopyrrin in subjects with unconjugated hyperbilirubinemia. In other words, bilirubin oxidation would be accelerated not only in the presence of oxidative injury or "stress", but also in response to the elevation of unconjugated serum bilirubin.

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**References**


