EFFECTS OF PHOTOTHERAPY ON HEPATIC FUNCTION IN HUMAN ALCOHOLIC CIRRHOSIS

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Phototherapy has been used to treat neonatal jaundice, but little assessment has been made of possible beneficial effects on adult liver disease. Effects of phototherapy on bile acid turnover, biliary lipid concentration, liver function tests, and bromosulfophthalein (BSP) kinetics were studied in 8 alcoholic cirrhotics. Phototherapy initially increased biliary specific activity of both primary bile acids and then produced an acceleration of cholic and chenodeoxycholic acid decay curves. Pruritus was relieved in the 3 patients who had this symptom. The proposed mechanism for these changes is mobilization of bile acids from an expanded cutaneous bile acid pool with augmented bile acid excretion. No significant change in serum liver function tests or BSP plasma disappearance curves was seen. Phototherapy causes little improvement in intrinsic liver function, but produces specific changes in bile acid metabolism; these changes may be related to effects of light on a cutaneous bile acid pool.

Since the demonstration in controlled studies that the degree of neonatal hyperbilirubinemia was lessened by light treatment, phototherapy has become a well recognized mode of treatment for neonatal jaundice. Hyperbilirubinemia is decreased by photodegradation of bilirubin to polar derivatives and augmented excretion of unconjugated bilirubin by an as yet undefined mechanism. Previous human studies have neither assessed the effects of phototherapy on other parameters of liver function nor have they investigated the possibility that phototherapy might alter excretion of organic anions other than bilirubin and produce intrinsic improvement in liver function. With these considerations in mind, the effects of phototherapy on bile acid kinetics, bile composition, serum liver function tests, and bromosulfophthalein (BSP) kinetics have been examined in 8 jaundiced patients with biopsy-documented alcoholic cirrhosis.

**Experimental Design**

Eight male patients, 4 Caucasians and 4 blacks, were studied. Informed consent was obtained from each subject. Ages ranged from 27 to 56 years. All had stable liver function tests for 1 week before investigation, and no patient had significant ascites. Phototherapy was provided by four high intensity Daylite fluorescent bulbs (General Electric Co., 40 w Power-Groove Lamps, cat. no. F48P-G-17D) mounted in two twin lamp reflectors (Miller Co., Meriden, Conn., no. IM-2501-04) that delivered 1000 foot-candles of illumination at the body surface.

Patients received a 2000-cal diet containing 100 g of fat, 80 g of protein, and 200 g of carbohydrate. Each patient ate three meals and an evening snack daily. The meal pattern and caloric distribution remained constant throughout the study. On the 1st day a small radiopaque nasoduodenal tube was passed, and its position in the third portion of the duodenum was verified fluoroscopically. Fifty microcuries of $^3$H-
chenodeoxycholic acid (CDC) and 10 $\mu$C of $^{14}$C-cholic acid (New England Nuclear, Boston, Mass.) were administered intravenously. Fasting AM and PM bile samples were aspirated daily before the morning and evening meals after cholecystokinin-stimulated gallbladder contraction was induced by intraduodenal instillation of amino acids (FreAmine, McGaw Laboratories, Glendale, Calif.). After a 3-day control period, patients remained continuously under the light for 72 hr, wearing short pants to maximize light exposure and dark glasses for eye protection. This was followed by a recovery period of 2 to 3 days with the lights off. No visible cutaneous hyperemia was noted in any patient during phototherapy, and surface temperature of skin measured in the last 2 patients studied did not change. At the end of each period, liver function tests were drawn and plasma BSP disappearance curves were obtained using a single intravenous injection of 5 mg per kg. Conjugated bile acids were enzymatically hydrolyzed by cholyglycine hydrolase (Schwarz-Mann, Worthington, N. J.). The deconjugated bile acids were extracted into ether and separated by ascending thin layer chromatography on 5- by 40-cm plates of silica gel 7G (A. H. Thomas, Philadelphia, Pa.). Iso-octane-isopropyl ether-glacial acetic acid (2:1:1 by volume) was the developing solvent (C. N. Williams and J. R. Senior, method unpublished). The interval between CDC and deoxycholic acid under these conditions is greater than 1 cm, and chromatography of radiolabeled standards of CDC and...
deoxycholic acid revealed greater than 96% separation of the two bile acids. Cholic and CDC spots were eluted with methanol and specific activities were determined by radioactive counting and hydroxysteroid dehydrogenase mass measurements. Bile acids were dissolved in 3 ml of ethanol and radioactivity was determined in a Beckman LS-200B liquid scintillation system after addition of 10 ml of toluene containing 8 g of 2,5-diphenyloxazole per liter. Both external standard ratios and internal standardization were used to correct for quenching. Bile acid kinetics were analyzed by the method of Lindstedt. Specific activity decay curves before and during phototherapy were calculated using the least squares linear regression; fractional turnover rates before and during phototherapy were compared using a paired t-analysis. Three morning bile specimens from each study period were analyzed for total bile acids, phospholipids, cholesterol, total bilirubin, and unconjugated bilirubin. Serum alkaline phosphatase, SGOT, albumin, and total serum proteins were measured by standard laboratory methods. Plasma BSP was measured using the method of Seligson et al., and compartmental analysis of the BSP plasma disappearance curves was performed by computer. Paired t-analysis was used for comparison of parameters before, during, and after phototherapy.

Results

Bile acid turnover and biliary lipid excretion. During the control period, the specific activity curves for both cholic and CDC acids showed the expected linear semilogarithmic decay described by Lindstedt. Pool sizes, fractional turnover constants, and half-lives determined from these initial decay curves are shown for both bile acids in table 1.

The decay curves deviated from linearity after the onset of phototherapy. An increase in specific activity from the previous value on the decay curve was consistently seen for both bile acids in the first bile specimen obtained after the onset of phototherapy. This rise in specific activity was followed by an accelerated rate of decline in specific activity which usually continued into semilogarithmic decay described by Lindstedt. Pool sizes, fractional turnover constants, and half-lives determined from these initial decay curves are shown for both bile acids in table 1.

Table 1. Bile acid kinetics in alcoholic cirrhosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pool size (amoles)</th>
<th>Turnover constant (days⁻¹)</th>
<th>Half-life (days)</th>
<th>Pool size (amoles)</th>
<th>Turnover constant (days⁻¹)</th>
<th>Half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. N.</td>
<td>1110</td>
<td>0.101</td>
<td>6.9</td>
<td>1200</td>
<td>0.099</td>
<td>7.0</td>
</tr>
<tr>
<td>J. F.</td>
<td>610</td>
<td>0.146</td>
<td>4.8</td>
<td>940</td>
<td>0.192</td>
<td>3.6</td>
</tr>
<tr>
<td>J. M.</td>
<td>286</td>
<td>0.148</td>
<td>4.7</td>
<td>486</td>
<td>0.250</td>
<td>2.8</td>
</tr>
<tr>
<td>S. O.</td>
<td>1453</td>
<td>0.088</td>
<td>7.9</td>
<td>1827</td>
<td>0.110</td>
<td>6.3</td>
</tr>
<tr>
<td>C. G.</td>
<td>597</td>
<td>0.105</td>
<td>6.6</td>
<td>2244</td>
<td>0.070</td>
<td>9.9</td>
</tr>
<tr>
<td>L. L.</td>
<td>1200</td>
<td>0.115</td>
<td>6.0</td>
<td>1456</td>
<td>0.150</td>
<td>4.6</td>
</tr>
<tr>
<td>J. H.</td>
<td>711</td>
<td>0.049</td>
<td>14.3</td>
<td>460</td>
<td>0.097</td>
<td>7.2</td>
</tr>
<tr>
<td>J. B.</td>
<td>655</td>
<td>0.126</td>
<td>5.5</td>
<td>3534</td>
<td>0.126</td>
<td>5.5</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>902 ± 208</td>
<td>0.110 ± 0.032</td>
<td>7.1 ± 1.1</td>
<td>1581 ± 358</td>
<td>0.137 ± 0.021</td>
<td>5.8 ± 0.8</td>
</tr>
</tbody>
</table>

To determine the magnitude of the specific activity increase, control decay curves were extrapolated forward to the time when the first phototherapy bile samples were obtained and the predicted bile acid specific activities were compared with the bile acid specific activities actually obtained experimentally (fig. 2). Paired t-analysis showed that actual bile acid specific activities obtained experimentally in initial phototherapy bile samples were significantly higher than those predicted by continued linear decay of the control period curves. Changes in slope of the specific activity decay curves were usually more prominent for cholic acid than for CDC (fig. 3), but a paired t-analysis comparing slopes of control and phototherapy decay curves showed a statistically significant increase in slope during phototherapy for both bile acids ($P < 0.01$ and $P < 0.05$, respectively).

Although all bile specimens during control, phototherapy, and recovery periods showed generally low levels of bile acids and other biliary lipids as expected in patients with cirrhosis, a small but significant increase in bile acid concentration was noted during phototherapy (fig. 4). Biliary phospholipid concentrations also increased ($P < 0.02$), but no increase in cholesterol concentrations ($P < 0.20$) was seen.

Bilirubin metabolism. Although neither direct nor total serum bilirubin levels decreased with phototherapy, a marked decrease in cutaneous icterus occurred. Bile samples became noticeably darker, with a statistically significant increase in bile bilirubin concentration (fig. 5). Thin layer chromatography of both control and phototherapy bile samples revealed that over 98% of the bilirubin was conjugated. In addition, no new spectrophotometric peaks or thin layer chromatography spots were observed.

Hepatic function. No significant changes in serum alkaline phosphatase, SGOT, albumin, or total protein occurred from the control period to the end of the study. Plasma BSP disappearance was measured at the end of each study period in 5 patients. In every patient, the three disappearance curves obtained for that patient...
Fig. 1. Effect of phototherapy on turnover of $^{14}$C-cholic acid and $^3$H-chenodeoxycholic acid in patients with alcoholic cirrhosis. Labeled bile acids were administered intravenously at time 0, and 72 hr of continuous phototherapy were given during the light period. Specific activities were determined for bile acids extracted from duodenal bile aspirated before breakfast and dinner each day.

Fig. 2. Rise in bile acid specific activities after onset of phototherapy. Predicted values for bile acid specific activity were determined for the time that the first phototherapy bile sample was obtained by forward extrapolation of the control decay curves. Predicted and actual experimental values were compared by paired t-analysis.

Fig. 3. Effect of phototherapy on turnover of $^{14}$C-cholic acid (left) and $^3$H-chenodeoxycholic acid (right) in 8 alcoholic cirrhotic patients. The fractional bile acid turnovers during the control and light periods are compared for each patient. The paired t-test was used for statistical analysis.

Discussion

The abrupt increase in bile acid specific activity shortly after the onset of phototherapy and the accelerated decline in the specific activity decay curve during phototherapy strongly suggest that phototherapy is altering bile acid metabolism. These studies were conducted before the observations of biological exchangeability of $^3$H in randomly tritiated bile acid preparations, and loss of $^3$H from the randomly tritiated CDC probably had some effect on the calculated CDC specific activities and pool sizes. However, because relative changes in specific activity both before and during phototherapy were compared in each patient, errors in absolute specific activities of CDC caused by biological exchange of tritium are of less importance. In addition, despite the limitations of tritiated bile acid preparations, the data for CDC are similar to that obtained by Vlahcevic et al., who used $^{14}$C-CDC when studying a similar group of patients. The finding of no significant changes in liver enzymes or BSP kinetics from the control period to the end of the study indicates that the changes in bile acid metabolism were due to a specific effect of phototherapy and not to spontaneous improvement in alcoholic liver disease. They also suggest that phototherapy is neither beneficial nor detrimental to intrinsic hepatic function in adults with compromised livers.

Several mechanisms could explain the changes in bile acid kinetics seen with phototherapy. Light conversion of the labeled bile acids to derivatives that were not substrates for the hydroxysteroid dehydrogenase assay would explain the initial increase in bile acid specific
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Fig. 4. Effect of phototherapy on biliary bile acid concentrations. Each point represents the mean total bile acid concentration from three fasting AM duodenal bile samples. Comparison between control and light values was significant at the P < 0.05 level. No significant difference (NS) was seen between light and recovery values.

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Fig. 5. Effect of phototherapy on bile bilirubin concentrations. Each point represents the average value obtained for three fasting morning bile samples from the control, light, and recovery periods. Comparison between control and light values was significant at the P < 0.05 level. No significant difference (NS) was seen between light and recovery values.

activity. However, no new spots were seen on chromatography of bile acids extracted from bile during phototherapy, and no change in enzymatic reactivity or chromatographic behavior was noted after in vitro phototreatment of bile acids. Light production of inhibitors of the hydroxysteroid dehydrogenase reaction could also explain the increase in specific activity, but no inhibitors were found when extracts of phototherapy bile were added to standard bile acid solutions and the solutions were assayed.

A clinical observation made during the course of the study suggests a third possibility. One patient's pruritus disappeared during phototherapy, reappeared after cessation of light treatment, and was then again relieved by cholestyramine. Two additional patients had initial exacerbation of pruritus at the start of phototherapy, with relief during the 2nd day. Skin bile acids have long been implicated in the genesis of pruritus associated with hepatobiliary disease, and high levels of skin bile acids in patients with hepatobiliary disease have been demonstrated. More recently, direct application of crude bile or pure preparations of bile acids to skin has produced pruritus. Bilirubin photodegradation is felt to occur predominantly in the skin, and the observation that pruritus was relieved in the 3 patients who had that symptom suggests that phototherapy may also be affecting a skin bile acid pool.

This hypothesis implies that the impaired hepatic clearance of the intravenously administered labeled bile acids leads to deposition of the labeled bile acids in the skin. Mobilization of this highly labeled cutaneous bile acid pool into the plasma during phototherapy could explain the abrupt increase in biliary bile acid specific activity if this cutaneous pool turned over more slowly and were not in equilibrium with the enterohepatic pool. Such a disequilibrium has been identified for bilirubin in vivo skin epithelium preparations of rodents. Bilirubin already bound to the skin is released more slowly into bilirubin-free incubation media than the rate of accumulation when fresh epithelial preparations are incubated with bilirubin-containing solutions. In addition, when data obtained in studies of bilirubin kinetics in humans were subjected to computer analysis using a multicompartamental model, the rate of flux of bilirubin from plasma into the extrahepatic extravascular pool was 3 times the rate of flux of bilirubin from that pool back into plasma. The finding of increased concentrations of conjugated bilirubin in bile during phototherapy fits with this hypothesis of an alteration in skin-binding
properties for organic anions during phototherapy. Based on estimated extracellular fluid volumes in their cirrhotic patients, Vlahcevic et al. have estimated that lack of miscibility between systemic and enterohepatic bile acid compartments might cause underestimation of the total bile acid pool size by 10 to 20% when the isotope dilution technique is used. Further disequilibrium between an additional tissue bile acid pool and the enterohepatic bile acid pool would raise questions concerning the validity of the Lindstedt method of determining bile acid kinetics in patients with liver disease.

Several mechanisms may contribute to the accelerated bile acid specific activity decline during phototherapy. While only bile acid concentrations, rather than total bile acid outputs, were directly measured, an increased biliary excretion of bile acids could result in increased fecal loss and explain the accelerated rate of specific activity decline. A rise in serum bile acids secondary to mobilization from skin into the circulation would increase the load of bile acids delivered to the kidney. Because bile acid renal clearance is directly proportional to the concentration of unbound plasma bile acids, saturation of albumin-bile acid bonding or a change in the properties of these bonds attributable to phototherapy would increase urinary bile acid excretion and accelerate specific activity decline.

Further studies are needed in which serum, urine, and skin radioactivity and bile acid levels are monitored during phototherapy in order to determine the direction and magnitude of change in these various bile acid pools. Presently attempts are underway to monitor directly these variables during phototherapy and to define the influence of phototherapy on the kinetics of bile acid-albumin bonding. Although these further studies are needed to confirm this hypothesis, the changes in bile acid kinetics noted with phototherapy provide a possible explanation for the old clinical observation that patients with chronic cholestatic jaundice itch less in the summer months. Phototherapy may be a useful adjunct in the treatment of pruritus associated with chronic hepatobiliary disease.

REFERENCES