Note

**Decline in Plasma Retinol in Unconjugated Hyperbilirubinemia
Treated with Bilirubin Adsorption Using an
Anion-Exchange Resin**

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(Received September 6, 1997)

Summary When an anion-exchange resin column (Plasorba BR-350) was used for the treatment of unconjugated hyperbilirubinemia, we found an unexpected decrease in plasma retinol (vitamin A) concentrations in a patient with type I Crigler-Najjar syndrome. The purpose of our study was to investigate the mechanism of this decrease in plasma retinol. When the patient's serum bilirubin exceeded the bilirubin binding capacity of 14.7 μmol bilirubin/g serum albumin (i.e., 720 $\mu\text{mol/L}$ of bilirubin), abrupt deterioration of the patient's neurologic status (suppression in his gait and speech) occurred, so the need to apply plasmapheresis to reduce the unconjugated bilirubin was indicated. Blood was drawn from the radial artery at a flow rate of 160 mL/min and pumped into a membrane plasma separator at a rate of 40 mL/min. The plasma was passed through the bilirubin adsorbent column and returned to the venous blood line of the plasma separator. Plasma samples were taken at the inlet and outlet of the bilirubin adsorbent column before and after treatment. The concentration of unconjugated bilirubin in plasma was effectively reduced by the perfusion, but plasma retinol was coincidentally decreased by the perfusion to vitamin A deficiency levels. The patient's plasma retinol was 2,127 nmol/L at the beginning of therapy and decreased to 1,492 nmol/L after repeated adsorption treatments. As the amounts of decrease in retinol (912 ± 123 nmol/L) after the perfusion were almost equal to those in retinol-binding protein ($1,010 \pm 192$ nmol/L), retinol may have been removed as a form of holo retinol-binding protein. Decreases in retinol and retinol-binding protein levels were also observed in low-density lipoprotein (LDL) apheresis with a dextran sulfate column (i.e., a cation-exchange resin column). In the patient with Crigler-Najjar

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syndrome, retinol taken dietarily was removed by plasmapheresis. However, the patient manifested no clinical symptoms associated with vitamin A deficiency, since his liver storage of retinol could supply the loss caused by plasmapheresis treatment. We should measure plasma retinol concentrations to evaluate the loss of retinol during plasmapheresis treatment coupled with an anion-exchange resin column.

Key Words plasma perfusion, bilirubin, retinol, vitamin A, anion-exchange resin

Plasma perfusion using an anion-exchange resin has been widely applied, and the effectiveness of the reduction of bilirubin for the treatment of hyperbilirubinemia has been described (1, 2). Despite a marked decrease in plasma bilirubin by the column, no significant loss of patient's plasma constituents such as plasma proteins were documented (2, 3). However, during therapeutic plasmapheresis to remove unconjugated bilirubin using an anion-exchange resin for a patient with severe unconjugated hyperbilirubinemia, we found a simultaneous decrease in plasma retinol (vitamin A). Although bilirubin was effectively removed from the plasma, the patient's plasma retinol concentration was decreased from ca. 2,127 to 1,492 nmol/L when plasmapheresis treatment had been performed for four mo. As vitamin A is one of the essential vitamins, we designed the following studies on an anion-exchange column to investigate the mechanism of the removal of retinol during plasmapheresis treatment.

Patient and methods

Patient. The patient was a 16-year-old Japanese adolescent (weight 77 kg, height 169 cm) who showed unconjugated hyperbilirubinemia soon after birth. He was diagnosed as having type I Crigler-Najjar syndrome on the basis of a complete absence of hepatic bilirubin UDP glucuronyltransferase (EC 2.4.1.17) activity, found at the age of 51 d. The administration of phenobarbital and cholestyramine did not decrease his serum bilirubin concentrations; only phototherapy was effective and prevented kernicterus. Since a majority of the patients with this disease eventually show central nervous system damage by hyperbilirubinemia, the patient had been treated with phototherapy every night for more than 10 h. However, his serum bilirubin concentrations continued to increase year after year. When the serum bilirubin exceeded the bilirubin binding capacity of 14.7 μ mol bilirubin/g serum albumin (i.e., 720 μ mol/L bilirubin), an abrupt deterioration of the patient's neurologic status occurred as manifested by difficulty in walking and speaking. At this point, plasmapheresis was conducted.

Methods. Plasmapheresis was carried out twice a week for a total of five mo. The following studies were performed when plasmapheresis treatment had been completed for four mo. After that, the patient received a liver transplant in Australia. In accordance with the Helsinki Declaration, informed consent was

strictly observed and the study design was approved by the Protection of Human Subjects Committee of Toho University.

In the plasmapheresis, a bilirubin adsorbent column (2) (Plasorba BR-350, Asahi Medical, Tokyo, Japan) was used to remove bilirubin from the plasma (Fig. 1). Blood was drawn from the radial artery at a flow rate of 160 mL/min and pumped into a membrane plasma separator (Plasmacure PS-06, Kuraray, Okayama, Japan). The yield of this separation was 40 mL/min. The plasma was then perfused through the bilirubin adsorbent column and returned to the venous blood line of the plasma separator. The bilirubin adsorbent material consisted of a strong-base, anion-exchange resin (styrene-divinyl benzene) in chloride form on a cellulose acetate filter with a 0.45 μm pore size. During the perfusion, sodium heparin was added to the blood in the separator at a rate of 1,000 U/h. The total volume of plasmapheresis was 7 L. An aliquot of plasma was collected every liter of plasmapheresis at the inlet and outlet of the adsorbent system (Fig. 1, sites Pads and Pv). The concentrations of unconjugated bilirubin, retinol and other micronutrients were determined for four perfusions performed on four successive days. Plasma concentrations of retinol, retinyl palmitate, α -tocopherol, and α and β carotenes were determined by a silver-ion-free HPLC method as described earlier (4). Various plasma constituents in arterial blood specimens collected at the inlet of the plasma separator (Fig. 1, site Pa) before and after 7-L plasmapheresis performed on another five successive days were measured with a Hitachi 7250 analyzer (Hitachi, Tokyo, Japan) using commercial test reagents (Dia-Iatron, Tokyo, Japan). Globulins were obtained by subtracting albumin concentrations from total protein concentrations. Plasma retinol-binding protein (RBP) was measured by the immunonephelometric method (5). Low-density lipoprotein (LDL) was measured by the heparin/ Ca^{2+} turbidimetric method (6). Differences between paired results were statistically examined by paired *t*-test.

Results and discussion

Preliminarily, we measured the plasma retinol and albumin-free bilirubin concentrations to confirm that these neurologic symptoms were not due to hypervitaminosis A toxicity but rather to high bilirubin, since retinoic acid (a metabolite of retinol) cannot be excreted in bile as a glucuronide conjugate in type I Crigler-Najjar syndrome (7). His plasma concentration of retinyl palmitate was 39 nmol/L (reference range: 0–191 nmol/L) at the plasma retinol concentration of 2,127 nmol/L (reference range: 1,050–2,800 nmol/L), but the albumin-free bilirubin concentration as determined with the peroxidase oxidation method (8) was 24 nmol/L (reference range: $\sim$ 17 nmol/L), indicating a likely progression of bilirubin encephalopathy. Hypervitaminosis A (vitamin A toxicity) is associated with increased plasma concentrations of retinyl esters (particularly, of retinyl palmitate) (9).

First, arterial blood specimens were collected at the inlet of the plasma separator before and after 7-L plasmapheresis on five successive days (Fig. 1, site Pa). The

Fig. 1. System of bilirubin adsorption. Plasma samples were collected at the inlet and outlet of the bilirubin adsorbent column (Plasorba BR-350, Asahi Medical; sites Pads and Pv, respectively). Blood specimens were collected at the inlet of the plasma separator (Plasmacure PS-06, Kuraray; site Pa) before and after 7 L of plasmapheresis.

Table 1. Analysis of plasma constituents^a before and after plasmapheresis treatment in a patient with type I Crigler-Najjar syndrome.

	Perfusion	
	Before	After (difference)
Protein		
Total (g/L)	67 ± 1	61 ± 1 ^b
Albumin (g/L)	48 ± 1	43 ± 1 ^b
Globulins (g/L)	19 ± 1	18 ± 1 ^c
Uric acid (mg/L)	64 ± 3	53 ± 2 ^b
Cholinesterase (IU/L) ^e	143 ± 12	108 ± 17 ^b
Bilirubin (μmol/L)	291 ± 36	166 ± 19 ^d
Retinyl palmitate (nmol/L) ^f	47 ± 19	23 ± 9 ^c
Retinol (nmol/L)	1,441 ± 104	529 ± 65 ^d (912 ± 123)
RBP (nmol/L)	1,686 ± 217	676 ± 71 ^d (1,010 ± 192)

^a Arterial blood specimens were collected at the inlet of the plasma separator before and after plasmapheresis on five successive days. Values are means and SD of five perfusions.

^b Statistically different from the before-perfusion values at $p < 0.01$ (paired t -test).

^c Statistically different from the before-perfusion values at $p < 0.05$ (paired t -test).

^d Statistically different from the before-perfusion values at $p < 0.001$ (paired t -test).

^e Reference value: 185–431 IU/L.

^f Reference value: 0–191 nmol/L.

data in Table 1 show that the concentration of unconjugated bilirubin in plasma was significantly reduced by the perfusion ($p < 0.001$). We did not measure the change in conjugated bilirubin because its concentration in the plasma of our patient was essentially zero. The plasma concentrations of sodium, potassium, chloride, calcium, inorganic phosphorous, magnesium, iron, urea nitrogen, creatinine, amylase, creatine kinase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyltransferase, glucose, triglycerides and total cholesterol did not change by the treatment using anion-exchange resin (data not shown), and our findings agree with those in previous reports (2, 3). Incompatible with the previous reports, plasma concentrations of total protein, albumin, uric acid and plasma cholinesterase activity were significantly reduced by the perfusion, and this needs further investigation. However, it is important to note that concentrations of RBP were also deleted by the perfusion. The decreased amounts of retinol (912 ± 123 nmol/L) were almost equal to those of RBP ($1,010 \pm 192$ nmol/L). Geiger et al (2) reported the significant loss of other plasma constituents such as complements (C_3 and C_4), immunoglobulins (Ig A, Ig G and Ig M), transferrin, α_1 -antitrypsin, α_1 -glycoprotein, α_2 -macroglobulin, haptoglobin and ceruloplasmin. Despite this, we observed a significant loss of globulins (i.e., sum of the above proteins), and post-perfusion values were within normal range (Table 1).

Next, we closely investigated the concentration of retinol in each liter of plasma taken at the inlet and outlet of the bilirubin adsorbent column (Fig. 1, sites Pads and Pv). Table 2 also displays that the concentration of retinol was significantly decreased to about 50% of the value before perfusion by perfusion through the

Table 2. Effect of plasma perfusion^a using an anion-exchange resin on the concentrations of bilirubin and retinol in a patient with type I Crigler-Najjar syndrome.

	Bilirubin (μ mol/L)		Retinol (nmol/L)	
Before perfusion	308 ± 32^b		$1,492 \pm 138^b$	
Perfusion (mL)	at inlet	at outlet	at inlet	at outlet
1,000	214 ± 36	90 ± 19 (42 ± 2) ^c	952 ± 162	484 ± 82 (51 ± 4) ^c
2,000	200 ± 37	106 ± 23 (53 ± 5)	882 ± 207	597 ± 201 (66 ± 10)
3,000	183 ± 21	115 ± 19 (63 ± 4)	758 ± 133	565 ± 145 (74 ± 11)
4,000	181 ± 20	118 ± 15 (65 ± 2)	764 ± 163	545 ± 81 (72 ± 7)
5,000	171 ± 22	123 ± 13 (72 ± 2)	647 ± 205	469 ± 167 (72 ± 8)
6,000	168 ± 24	122 ± 16 (73 ± 2)	640 ± 62	501 ± 8 (79 ± 8)
7,000	169 ± 19	126 ± 14 (75 ± 2)	652 ± 95	520 ± 74 (80 ± 8)
After perfusion	173 ± 16^b		737 ± 55^b	

^a Values are means and SD of four perfusions performed on four successive days.

^b Values in blood collected at the inlet of a membrane plasma separator.

^c Rates of removal (%) were calculated as (concentration after perfusion at outlet of the resin column)/(concentration before perfusion at inlet of the resin column).

anion-exchange resin. In the first liter of perfusate, 58% of the bilirubin was adsorbed while only 49% of the retinol was removed ($p < 0.01$); however, no significant differences in the removal of these analytes were found at 3 and 7 L of perfusion. On the four successive days when perfusion was performed, the concentration of retinol before perfusion was 1,668 on day 1, 1,338 on day 2, 1,446 on day 3 and 1,516 nmol/L on day 4. The mean ($\pm$ SD) retinol was $1,492 \pm 138$ nmol/L; these decreased to 784, 770, 731 and 662 nmol/L following perfusion on the four successive days (mean $\pm$ SD, 737 ± 55 nmol/L). Although plasma retinol after perfusion was decreased to vitamin A deficiency levels [$< 1,050$ nmol/L (7)], it returned to normal on the next day; presumably stored retinol in the liver was secreted into the circulation. Since humans have a large capacity for storing retinol in the liver (10) and the turnover of RBP is extremely fast (11, 12), symptoms associated with hypovitaminosis A were not manifested in our patient. We work out ca. 4,000 mL of circulating plasma volume in the patient from his body weight (77 kg) and venous hematocrit value (37%). Thus, maximally 3,600 nmol of retinol will be lost per d, since 500–900 nmol/L of retinol was deleted by 7 L of plasmapheresis. As studies on daily food intake revealed that the patient took more than 3,000 IU ($\approx 3,490$ nmol) of retinol from the diet per d at that time, the losses by plasmapheresis were as much as the daily oral intake of retinol. Accordingly, daily requirement should be provided with hepatic vitamin A stores. Here, the requirements (1,971 nmol) were based on the order of 22 IU (25.6 nmol) per kilogram per body weight per d (13). We also calculated how many times it takes for perfusion to exhaust liver storage. The average adult will have 250 nmol of retinol in each gram of liver (13). The patient's hepatic vitamin A stores would be estimated at 442,750 nmol from his liver weight of 1,771 g (2.3% of the body weight). Our patient may have a 224-d supply of retinol in the liver, if the plasmapheresis treatment is performed every day. We should measure plasma retinol concentrations during plasmapheresis treatment coupled with an anion-exchange resin column to estimate the loss of retinol.

In the absence of the anion-exchange resin, bilirubin (2) and retinol were not removed by the membrane plasma separator, most likely because unconjugated bilirubin is tightly bound to albumin (3), and retinol is bound to both RBP and prealbumin (7). The addition of anion-exchange resin to the system makes the removal of bilirubin from plasma possible. The removal mechanism is likely anion exchange: the resin binds the carboxylic acid groups of bilirubin (COO), and releases Cl ions from the resin (14). Although retinol does not have carboxylic acid groups, it binds to the resin (presumably as a form of holo-RBP) owing to the strong hydrophobic character of both resin and retinol. We found that Cl concentrations were unaffected and remained stable throughout the perfusion. Furthermore, the concentrations of retinyl palmitate (an ester of retinol), α -tocopherol and carotenes did not change significantly. In a future study, we plan to test whether other anion-exchange resins (15) (e.g., weak-base resins) are also capable of adsorbing retinol along with bilirubin during plasmapheresis for hyperbilirubinemia. We

Table 3. Plasma retinol and RBP levels^a before and after LDL apheresis for three familial hypercholesterolemic patients.

	LDL apheresis	
	Before	After
LDL (mg/L) ^b	10,624 ± 1,677	2,236 ± 483 ^c
Retinol (nmol/L)	1,993 ± 164	1,615 ± 388 ^d
RBP (nmol/L)	1,889 ± 345	1,413 ± 225 ^d
Retinol/RBP ratios	1.11 ± 0.18	1.14 ± 0.28

^a Values are means and SD of three duplicate analyses.

^b Reference value: 1,880–4,920 mg/L.

^c Statistically different from the before-perfusion values at $p < 0.001$ (paired *t*-test).

^d Statistically different from the before-perfusion values at $p < 0.05$ (paired *t*-test).

measured plasma retinol levels in three cases of familial hypercholesterolemia treated with LDL apheresis by passage through a dextran sulfate column (a cation-exchange resin column; Kaneka Liposorber LA15 System, Tokyo, Japan) (16). When 4,000 mL of plasma were treated, retinol levels were significantly decreased (Table 3). RBP levels were also reduced, exhibiting similar retinol/RBP ratios before and after LDL apheresis. This suggested that decreases in retinol concentration would also be reflected by RBP levels. Here, RBP would be protonic, since proteins are "zwitterions."

In this investigation, we found that unconjugated bilirubin was removed from plasma of a patient with Crigler-Najjar syndrome by plasmapheresis and passage of the plasma through an anion-exchange resin. Our result is based on only one case; nevertheless, it is important because few patients with type I Crigler-Najjar syndrome live without treatment such as plasmapheresis, since liver transplantation is ethically unusual in Japan. During this therapy, which was performed twice a week for five mo, there was a temporary decrease in the concentration of plasma retinol, which returned to normal the day after plasmapheresis. The decrease was associated with the reduction of plasma retinol-binding protein. The patient may have sufficient supply of retinol in the liver. The return of the plasma retinol level to normal the day after plasmapheresis makes the possibility of vitamin A deficiency highly improbable.

The authors are particularly indebted to Dr. John A. Lott, Professor of Pathology, Ohio State University Medical Center, for his help in the preparation of this paper and the many useful suggestions for its improvement. The authors are also indebted to Mr. Kazuhiro Kunimi and the Clinical Chemistry Laboratory at Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. for assistance with the retinol-binding protein measurement.

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