Effects of Percutaneous Transhepatic Biliary Drainage on Blood–Bile Permeability and Selective IgA Transport in Patients with Biliary Obstructions

GAKUJI OHSHIO, M.D., TADAO MANABE, M.D., KOICHIRO TAMURA, M.D., HIROYUKI KUDO, M.D., HIDEYUKI YOSHIOKA, M.D., and TAKAYOSHI TOBE, M.D.

Biliary obstruction induces a increase in the permeability between blood and bile, and a decrease in the rate of immunoglobulin A (IgA) transport into bile. We investigated the effects of percutaneous transhepatic biliary drainage (PTBD) on these derangements. PTBD reduced the extent of elevation of the bile-to-serum ratio of Immunoglobulin G (IgG; IgG-BS ratio) in patients with obstructive jaundice. Because IgG is known to be passively transported from serum to bile, the results indicate that PTBD restores the blood–bile barrier function. The IgA-BS ratio/IgG-BS ratio index (IgA/IgG index) and the IgM/IgG index, which indicated the function of selective transport of IgA and IgM into bile, initially decreased and then returned to the normal range 17 days after PTBD in patients who experienced a rapid resolution of hyperbilirubinemia. However these indices remained low in patients who did not experience this resolution. The serum secretory IgA levels in patients who did not experienced rapid resolution of hyperbilirubinemia markedly increased before PTBD. The serum secretory IgA levels in the patients who did and those who did not experience rapid resolution of hyperbilirubinemia, after initially increasing, decreased after PTBD. However the level returned to the control range only in patients who experienced a rapid resolution. These results indicate that the secretory IgA level is a sensitive indicator of hepatobiliary function, and measurement of the level of secretory IgA could predict the effect of PTBD.

From the Department of Surgery and Geriatric Medicine, Faculty of Medicine, Kyoto University, Kyoto, Japan

BILIARY OBSTRUCTION AND obstructive jaundice have many adverse effects on liver, cardiovascular, renal, and immunologic functions. Biliary obstruction induces morphologic disruption in the tight junction,[1] increased permeability between blood and bile, and destruction of the blood–bile (canaliculosinusoidal) barrier function.[2]

In rats ligation of the biliary tract induces elevation of serum levels of immunoglobulin A (IgA) and of secretory components.[3] Kloppel et al.[4] have reported that IgA transport into bile is depressed after temporary bile duct clamping. In humans obstructive jaundice is known to elevate serum levels of IgA, secretory IgA, and IgA-containing circulating immune complexes.[5,6] However the function of selective IgA transport into bile after decompression is unknown. In this communication we observed that the function of selective IgA transport into bile was recovered only in the patients who experienced rapid resolution of hyperbilirubinemia after percutaneous transhepatic biliary drainage (PTBD), whereas in all of the patients PTBD reduced the degree of blood–bile permeability elevation. In addition we found that serum secretory IgA level was a good marker of hepatobiliary function.

Address reprint requests to Gakuji Ohshio, M.D., Department of Surgery, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan.

Accepted for publication May 24, 1989.

Patients and Methods

Patients and Samples

From 43 patients undergoing PTBD before surgery for biliary obstruction due to biliary tract stones (n = 2) or to tumors of the biliary tract or of the pancreas (n = 41), 78 samples of serum and/or hepatic bile were obtained. The catheter was placed properly and continuous bile flow was observed in all patients. Patients with acute cholangitis or other complications were excluded from this study.

The patients with obstructive jaundice were divided into three groups. Group A: total serum bilirubin of more than 10 mg/dL before PTBD and less than 5 mg/dL 20 days after PTBD (n = 21); group B: total serum bilirubin of more than 10 mg/dL before PTBD and more than 5 mg/dL 20 days after PTBD (n = 15); and group C: total serum bilirubin of less than 10 mg/dL before PTBD (n = 7).

There were no significant difference in the levels of total serum bilirubin or of glutamic oxaloacetic transaminase (GOT) between group A and B before PTBD (Table 1).

A control group of patients, each of whom underwent a cholecystectomy and T-tube drainage (n = 8) at least 11 days earlier, were also studied. All the samples were collected in the morning after an overnight fast and stored at −70 C until analysis. The study was carried out in accordance with the Helsinki Declaration.

Measurement of IgA, IgG, and IgM Concentrations in Serum and Bile

The concentrations of IgA, immunoglobulin G (IgG), and immunoglobulin M (IgM) were measured by enzyme-immunoassays (MBL, Nagoya, Japan), as reported earlier.[2] To samples diluted with phosphate-buffered saline (PBS) were added anti-human IgA, IgG, and IgM antibodies, which adhered to polystyrene beads in microtubes. These combinations were incubated for 3 hours at 37 C. After washing with PBS, β-D-galactosidase-conjugated anti-human IgA, IgG, and IgM antibodies were added to the tubes, incubated for 2 hours at 37 C, and washed again. A substrate (O-nitrophenyl-β-D-galactopyranoside) was then added to the tubes. After incubation for about 18 hours at 37 C, the optical density (OD) was measured at 420 nm. The specificity of these assays was confirmed using purified IgA, IgG, and IgM (Cappel, Malvern, PA).

The concentrations of IgG in bile was expressed as the bile-to-serum (BS) ratio. To compare different proteins, the concentrations of IgA and IgM in the bile were expressed as the index of the bile-to-serum ratio of the respective immunoglobulin (BS ratio) to the BS ratio of IgG (IgA or IgM-BS ratio/IgG-BS ratio).

Measurement of Secretory IgA Levels in Serum

An enzyme immunoassay for secretory IgA (MBL) was used and has been reported earlier.[7] Diluted samples were reacted with goat anti-human free secretory component antibodies, which adhered to polystyrene beads in microtubes, for 1 hour at 37 C. After washing, peroxidase-conjugated $F(ab')_2$ goat anti-human IgA antibodies were added to the tubes and then incubated for 1 hour at 37 C. After washing, a substrate (0-phenylenediamine in 0.1 M phosphate buffer, pH 6.0, containing 0.03% hydrogen peroxide) was added to the tubes. After further incubation for 1 hour at room temperature, the optical density was measured at 492 nm. The specificity of these antibodies was confirmed using purified secretory IgA, IgA free of secretory component, and free secretory component (Cappel and MBL).

TABLE 1. *The Levels of Total Serum Bilirubin and GOT Before PTBD*

Group	Total Serum Bilirubin (mg/dL)	GOT (IU)
Group A	15.6 ± 1.2	138 ± 26
Group B	17.9 ± 1.1	101 ± 15
Group C	7.2 ± 0.4	192 ± 82

Group A: Total serum bilirubin more than 10 mg/dL before PTBD and <5 mg/dL 20 days after PTBD.

Group B: Total serum bilirubin more than 10 mg/dL before PTBD and >5 mg/dL 20 days after PTBD.

Group C: Total serum bilirubin less than 10 mg/dL before PTBD.

There was no significant difference between group A and group B (mean ± SEM).

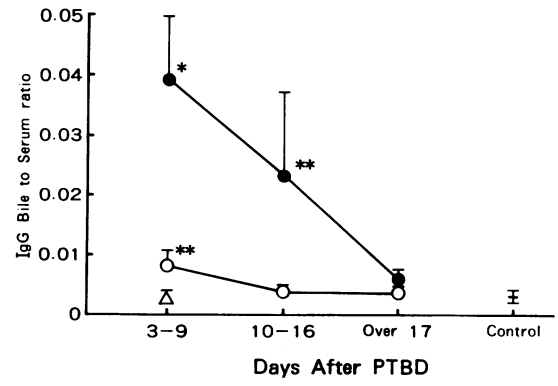

FIG. 1. The changes of the IgG bile-to-serum (BS) ratio of group A (open circle: patients who experienced a rapid resolution of hyperbilirubinemia), group B (closed circle: patients who did not experience a rapid resolution of hyperbilirubinemia), group C (open triangle: total serum bilirubin < 10 mg/dL before PTBD), and the control group (vertical line). *group B > group A and control, $p < 0.01$. **group A or B > control, $p < 0.05$. There is no significant difference between group C and the control group.

Statistical Analysis

Results were expressed as the mean ± standard error (SEM). Statistical analysis was performed by Wilcoxon's rank sum test,[8] and probability values (p values) less than 0.05 were considered significant.

Results

The BS ratio of IgG (IgG-BS ratio) in group A (rapid decrease in the total serum bilirubin level after PTBD) was high during the period between day 3 and day 9 but decreased to control levels during the period between day 10 and day 16 after PTBD (Fig. 1). In group B (no decrease in the total serum bilirubin level to less than 5 mg/dL), the ratio was markedly high during the period between day 3 and day 9 and also decreased but over a longer period of time. There were no significant difference in serum levels of IgA, IgG, and IgM between group A and B (Table 2). There was no significant difference between the IgG-BS ratio of group C (total serum bilirubin less than 10 mg/dL before PTBD) and that of the control group. Because it is known that IgG is passively transported from serum to bile,[9,10] the results indicate that the permeability increases in patients with obstructive jaundice and that these derangements are not due to artificial contaminations. PTBD reduces the degree of blood–bile

TABLE 2. *The Levels of IgA, IgG, and IgM in Serum and Bile After PTBD*

Group	Bile (mg/dL)			Serum (mg/dL)		
	IgA	IgG	IgM	IgA	IgG	IgM
Group A						
3 to 9 days	16.2 ± 5.1	24.0 ± 8.2	9.7 ± 4.2	609 ± 102	2417 ± 233	169 ± 18
10 to 16 days	9.9 ± 1.9	8.1 ± 1.8*	4.5 ± 1.1	546 ± 114	2555 ± 330	179 ± 14
More than 17 days	12.3 ± 25	7.1 ± 2.2*	3.3 ± 0.9*	561 ± 74	2439 ± 309	152 ± 21
Group B						
3 to 9 days	48.7 ± 17.6	97.9 ± 40.3	13.3 ± 4.9	602 ± 97	2282 ± 363	237 ± 65
10 to 16 days	45.1 ± 23.9	50.8 ± 31.2	3.6 ± 0.9*	642 ± 128	2374 ± 417	225 ± 39
More than 17 days	10.8 ± 3.8*	11.7 ± 4.6*	3.2 ± 1.0*	662 ± 146	1896 ± 159	196 ± 53
Group C						
3 to 9 days	14.1 ± 4.1	7.1 ± 2.2	6.8 ± 3.4	720 ± 104	2426 ± 325	139 ± 41

Group A: Total serum bilirubin more than 10 mg/dL before PTBD and less than 5 mg/dL 20 days after PTBD.

Group B: Total serum bilirubin more than 10 mg/dL before PTBD and more than 5 mg/dL 20 days after PTBD.

Group C: Total serum bilirubin less than 10 mg/dL before PTBD.

Control: More than 11 days after cholecystectomy and T-tube drainage.

* Significant difference between values at 10 to 16 days or more than 17 days and that at 3 to 9 days ($p < 0.05$). There was no significant differences in serum immunoglobulin levels among groups A, B, and C (mean ± SEM).

permeability elevation in both the patients who experienced a rapid decrease of total serum bilirubin and those who did not.

The changes of the IgA/IgG index (IgA-BS ratio/IgG-BS ratio) are shown in Figure 2. The IgA/IgG index of group A was slightly low during the period between day 3 and day 9 and increased to the normal range during the period between day 10 and day 16 after PTBD. On the contrary, the IgA/IgG index of group B was markedly low during the period between day 3 and day 9 and remained low even when measured 17 days after PTBD.

The changes of the IgM/IgG index are shown in Figure 3. The index of group B was also found to be low during the period between day 3 and day 9, and significantly lower than that of group A 17 days after PTBD. These results indicate that the ability to selectively transport IgA and IgM into bile was recovered only in those who experienced a rapid resolution of hyperbilirubinemia after PTBD.

The serum secretory IgA level before PTBD was significantly higher in group B than in group A (Fig. 4). The serum secretory IgA level in group A decreased to the control range after 17 days. In group B it decreased slightly after PTBD; however the level remained high even after 17 days.

Discussion

IgA and IgM are selectively transported into bile,[11,12] whereas most other proteins (*i.e.*, IgG and albumin) in bile are derived from circulating plasma by molecular weight-dependent passive transport.[9,10] We have reported that a increased BS ratio of IgG or albumin indicates an increase in permeability between blood and bile and a

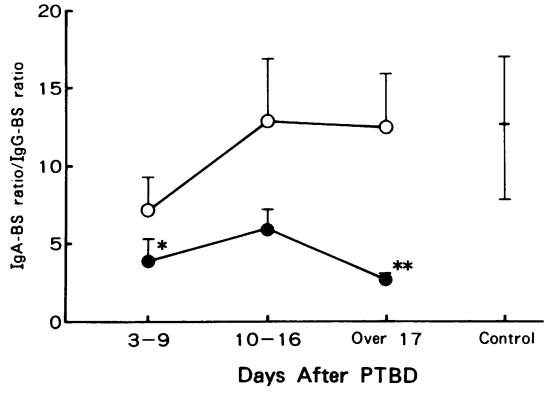

FIG. 2. The changes of the IgA/IgG index (IgA-BS ratio/IgG-BS ratio) of group A (open circle: patients who experienced a rapid resolution of hyperbilirubinemia), group B (closed circle: patients who did not experience a rapid resolution of hyperbilirubinemia), and the control group (vertical line). *group B < control, $p < 0.05$. **group B < group A and control, $p < 0.01$.

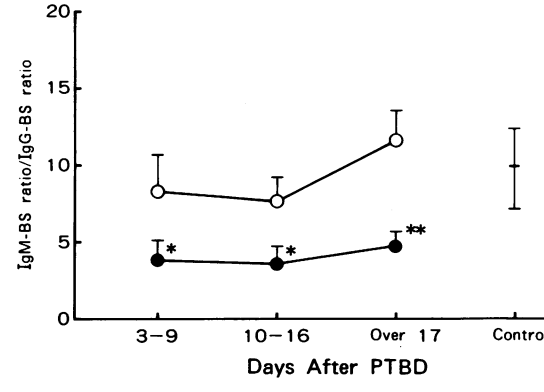

FIG. 3. The changes of the IgM/IgG index (IgM-BS ratio/IgG-BS ratio) of group A (open circle: patients who experienced a rapid resolution of hyperbilirubinemia), group B (closed circle: patients who did not experience a rapid resolution of hyperbilirubinemia), and the control group (vertical line). *group B < control, $p < 0.05$. **group B < group A, $p < 0.05$.

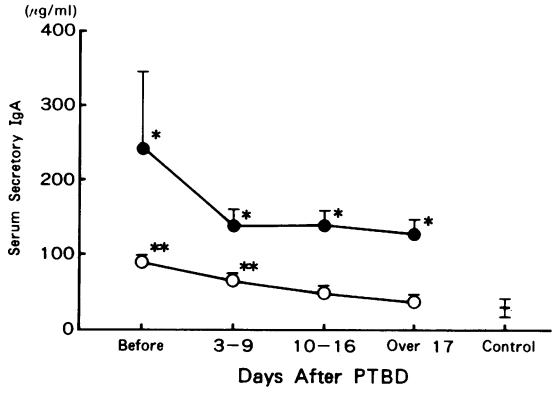

FIG. 4. The changes in serum secretory IgA levels of group A (open circle: patients who experienced a rapid resolution of hyperbilirubinemia), group B (closed circle: patients who did not experience rapid resolution of hyperbilirubinemia), and the control group (vertical line). *group B > group A, $p < 0.05$ and group B > control, $p < 0.01$. **group A > control, $p < 0.05$.

deterioration of the blood–bile barrier function in patients with biliary obstruction.[2] Tight junctions between hepatocytes have been recognized as the only structures responsible for the barrier between blood and the lumen of the bile canaliculus. Robenek et al.[1] have reported that extrahepatic biliary obstruction causes decontinuities in the junctional meshwork that provide a direct pathway between the lumen of the bile canaliculus and the intercellular space.

Our results showed that PTBD reduced the degree of blood–bile permeability elevation in patients with obstructive jaundice, although the time requirements were different for each group. Thus PTBD restores the function of the tight junction in patients with obstructive jaundice. Endotoxemia is one of the most common causes of complications and multiple-organ failure before and after an operation on patients with biliary obstruction. The levels of endotoxin in patients with gram-negative bacteria in bile show a greater increase than do those in patients with sterile cultures, and the destraction of the blood-bile barrier function might be a cause of endotoxemia.[13] The recovery of the blood-bile barrier function may have clinical significance.

The ability to selectively transport IgA and IgM into bile was recovered in those patients who experienced a rapid resolution of hyperbilirubinemia after PTBD but not in those patients whose total serum bilirubin level did not decrease to less than 5 mg/dL. There was no difference in serum immunoglobulin levels between these two groups. Serum immunoglobulins are one source of biliary IgA and IgM.[9–11] The cause of the failure to recover the ability to selectively transport is not due to the decreased levels of serum immunoglobulins.

The serum secretory IgA level before PTBD was significantly higher in those patients whose total serum bilirubin level did not decrease to less than 5 mg/dL than in patients who experienced a rapid resolution of hyperbilirubinemia. The serum secretory IgA level decreased initially after PTBD in both groups but returned to the control range only in patients who experienced a rapid resolution of hyperbilirubinemia. The increased level of serum secretory IgA found in conjuction with liver diseases is considered to be due to a reflux of secretory IgA and free secretory component from bile to serum and/or a direct release of free secretory component from hepatobiliary tissue.[14] The decrease in the serum secretory IgA level after PTBD may be due to a restoration of the function of the tight junction and decreased amount of reflux of secretory IgA and secretory component from bile to serum. Secretory IgA levels in patients who did not experience a rapid resolution of hyperbilirubinemia still remained high even at 17 days, while the permeability between serum and bile returned to the normal range. The cause of the increased secretory IgA levels in these patients is still unknown and needs further investigation.

It has been reported that PTBD improves liver, renal, and immunologic function and decreases the operative mortality rate in obstructive jaundice cases.[15–17] However a high drainage-related complication rate is also reported.[18–19] We have shown that the serum secretory IgA level is a sensitive indicator of hepatobiliary function and that the secretory IgA level before PTBD is related to the effect of PTBD. Measurement of the level of serum secretory IgA could predict the effect of PTBD.

References

1. Robenek H, Herwig J, Themann H. The morphologic characteristics of intercellular junctions between normal human liver cells and cells from patients with extrahepatic cholestasis. Am J Pathol 1980; 100:93–114.
2. Ohshio G, Furukawa F, Manabe T, et al. Comparative studies on immunoglobulins, complement component (C3), albumin, and immunoglobulin A-containing circulating immune complexes in serum and bile of patients with biliary obstruction. Dig Dis Sci 1988; 35:570–576.
3. Lemaitre-Coelho I, Jackson GDF, Vaerman JP. High levels of secretory IgA and free secretory component in the serum of rats with bile duct obstruction. J Exp Med 1978; 147:934–939.
4. Kloppel TM, Hoops TC, Gaskin D, Le M. Uncoupling of the secretory pathways for IgA and secretory component by cholestasis. Am J Physiol 1987; 256:G232–G240.
5. Ohshio G, Furukawa F, Sekita K, et al. IgA containing circulating immune complexes and IgA antisingle stranded DNA antibodies in patients with obstructive jaundice. Clin Exp Immunol 1985; 59:435–441.
6. Ohshio G, Furukawa F, Manabe T, et al. Relationship between secretory IgA, IgA containing (C3-fixing) circulating immune complexes and complement components (C3, C4) in patients with obstructive jaundice. Scan J Gastroenterol 1986; 21:151–157.
7. Ohshio G, Kudo H, Yoshioka H, et al. Plasma levels of secretory IgA in patients with gastric cancer. J. Cancer Res Clin Oncol 1987; 113:573–575.
8. Colten T. Statistics in Medicine. Boston: Little, Brown, 1974.
9. Dive C, Heremans JF. Nature and origin of the proteins of bile. I.

A comparative analysis of serum and bile proteins in man. Eur J Clin Invest 1974; 4:235–239.

10. Dive C, Nadalini RA, Vaerman JP, Heremans JF. Origin and nature of the proteins of bile. II. A comparative analysis of serum, hepatic lymph and bile proteins in the dog. Eur J Clin Invest 1974; 4: 241–246.
11. Delacroix DL, Hodgson HJF, McPherson A, et al. Selective transport of polymeric immunoglobulin A in bile. Quantitative relationships of monomeric and polymeric immunoglobulin A, immunoglobulin M, and other proteins in serum, bile, and saliva. J Clin Invest 1982; 70:230–241.
12. Kutteh WH, Prince SJ, Phillips JO, et al. Properties of immunoglobulin A in serum of individuals with liver diseases and hepatic bile. Gastroenterology 1982; 82:184–193.
13. Ohshio G, Manabe T, Tobe T, et al. Circulating immune complex, endotoxin, and biliary infection in patients with biliary obstruction. Am J Surg 1988; 155:343–347.
14. Delacroix DL, Reynaert M, Pauwels S, et al. High serum levels of secretory IgA in liver disease. Possible liver origin of the circulating secretory component. Dig Dis Sci 1982; 27:333–340.
15. Nakayama T, Ikeda A, Okuda K. Effect of percutaneous transhepatic drainage upon liver function and postoperative mortality. Surg Gynecol Obst 1982; 155:161–166.
16. Smith RC, Pooley M, George CRP, Faithful GR. Preoperative percutaneous transhepatic internal drainage in obstructive jaundice: a randomized, controlled trial examining renal function. Surgery 1985; 97:641–647.
17. Roughneen PTR, Gouma DJ, Kulkani AD, et al. Impaired specific cell-mediated immunity in experimental biliary obstruction and its reversibility by internal biliary drainage. J Surg Res 1986; 41: 113–125.
18. McPherson GAD, Benjamin IS, Hodgson HJF, et al. Pre-operative percutaneous transhepatic biliary drainage: the results of a controlled trail. Br J Surg 1984; 71:371–375.
19. Pitt HA, Gomes AS, Lois JF, et al. Does preoperative percutaneous biliary drainage reduce operative risk or increase hospital cost? Ann Surg 1985; 201:545–553.