

# Tissue Plasminogen Activator and Plasminogen Activator Inhibitor-1 in Human Choledochal Bile

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## Abstract

Fibrinolytic properties have been detected in animal and human gallbladder (GB) bile. Plasminogen activator inhibitor-1 (PAI-1) has been reported in greater concentration in GB stone bile and may be a nucleating factor in the pathogenesis of GB stone formation. It is unknown whether or not human choledochal bile has similar properties, which could have a role in choledocholithiasis. The aims of this study were to determine the presence of fibrinolytic properties of human choledochal bile and to compare those properties among normal, acalculous, and calculous-infected choledochal bile. Tissue plasminogen activator (t-PA) and PAI-1 of choledochal bile were measured by enzyme linked immunosorbent assay in patients with cholangitis due to acalculous bile duct obstructions (n=9), choledocholithiasis with cholangitis (n=20), and normal bile (n=7). The t-PA concentration of choledochal bile was no different among the three groups (acalculous-infected bile, median 4.61 ng/ml, and calculous-infected bile, 4.61 ng/ml, versus normal bile, 7.33 ng/ml). PAI-1 was detected in choledochal bile in significantly greater concentrations in patients with acalculous cholangitis due to bile duct obstructions and choledocholithiasis with cholangitis (acalculous-infected bile, median 0.36 ng/ml, and calculous-infected bile, 0.1 ng/ml, versus normal bile, 0.02 ng/ml,  $p < 0.05$ ), but the bile concentration of PAI-1 was no different between the acalculous and calculous-infected choledochal bile. Human choledochal bile possesses t-PA and PAI-1. PAI-1 was present in greater concentrations in both acalculous and calculous-infected choledochal bile. Increased levels of PAI-1 may be an epiphenomenon of cholangitis rather than a factor in the pathogenesis of choledocholithiasis.

**Key Words:** Fibrinolysis, biliary tract, choledocholithiasis

## INTRODUCTION

Fibrinolytic activity has previously been detected in animal bile and human gallbladder bile.<sup>1,2</sup> Plasminogen activator inhibitor-1 has been reported to be present in greater concentrations in GB stone bile and may be a nucleating factor in the pathogenesis of GB stone formation.<sup>3</sup> It is unknown whether human choledochal bile has fibrinolytic activity. We were

interested in determining whether human choledochal bile has similar properties, which could have a role in choledocholithiasis. The aims of this study were to investigate the presence of fibrinolytic proteins in human choledochal bile and to compare those properties among normal, acalculous-infected, and calculous-infected human choledochal bile.

## MATERIALS AND METHODS

### Patients

Thirty-six patients whose bile was collected at Yongdong Severance Hospital between July 1997 and June 1998, were included. The bile samples were obtained from three groups of patients: Those undergoing elective surgery for non-biliary diseases without clinical, biochemical, radiological or operative evidence of gall stones (Group 1); those having cholangi-

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tis with clinical evidence of right upper quadrant pain, fever, and jaundice, with positive bile culture due to acalculous bile duct obstruction without evidence of gall stone (Group 2); and those having cholangitis with clinical evidence and positive bile culture due to choledocholithiasis (Group 3). These patients in Group 3 were identified as having soft, light brown stones, which were characteristics of brown-pigmented bile duct stones, by endoscopic stone removal.

### Sampling of bile juice

Normal bile was aspirated from the biliary tree of those patients undergoing elective surgery for non-biliary disease by direct needle aspiration during the operation. Infected bile was aspirated from the bile duct of patients having cholangitis by endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC).

### Storage of bile juice

The aspirated and collected bile was stored in a Stabilyte™ tube (Biopool®, Umea, Sweden), in a freezer at  $-70^{\circ}\text{C}$  before assay.

### Assays of bile juice

Tissue plasminogen activator and plasminogen activator inhibitor-1 were measured by enzyme linked immunosorbent assay (ELISA) (TintElize, Porton Cambridge Ltd., Maidenhead, UK).

### Statistics

Statistical analysis was performed with the Kruskal-Wallis test and values of  $p < 0.05$  were considered to be significant.

## RESULTS

Group 1: Normal bile was aspirated from the biliary tree of seven patients (three female; mean age 55.5 years) who underwent elective laparotomy for non-biliary diseases. Group 2: infected, acalculous choledochal bile was obtained from nine patients (three female; mean age 65.3 years) of bile duct obstruction with clinical and bacteriological evidence of cholangitis, and without evidence of gall stones. Group 3: infected calculous choledochal bile was obtained from 20 patients (15 female; mean age 64.0 years) of choledocholithiasis with clinical and bacteriological evidence of cholangitis (Table 1).

Tissue plasminogen activator and plasminogen activator inhibitor-1 were present in the human choledochal bile.

There was no statistical difference in the choledochal bile concentration of tissue plasminogen activator among the three groups (acalculous-infected bile (group 2), median 4.61 ng/ml and calculous-infected bile (group 3), 4.61 ng/ml versus normal bile (group 1), 7.33 ng/ml) (Fig. 1).

Plasminogen activator inhibitor-1 was detected in choledochal bile in significantly greater concentrations in patients with acalculous cholangitis due to bile

Table 1. Clinical Characteristics of Patients

	Normal bile G1 (n=7)	Acalculous G2 (n=9)	Calculous G3 (n=20)	P-value ( $<0.05$ )
Age (years)	55.5	65.3	64.0	
Male : Female	4 : 3	6 : 3	5 : 15	c
WBC (1,000/ul)	7127.1	8385.6	12211.1	
T.bil (mg/dl)	1.2	9.1	3.7	a
ALP (U/L)	82.3	502.7	310.1	a, b
AST (IU/L)	19.6	95.1	135.1	a, b
ALT (IU/L)	20.0	143.8	184.4	a, b
t-PA (ng/ml)	8.4	10.8	6.0	
PAI-1 (ng/ml)	0.02	1.12	1.24	a

T.bil, total bilirubin.

Mean, a: G1 vs G2; b, G1 vs G3; c, G2 vs G3.

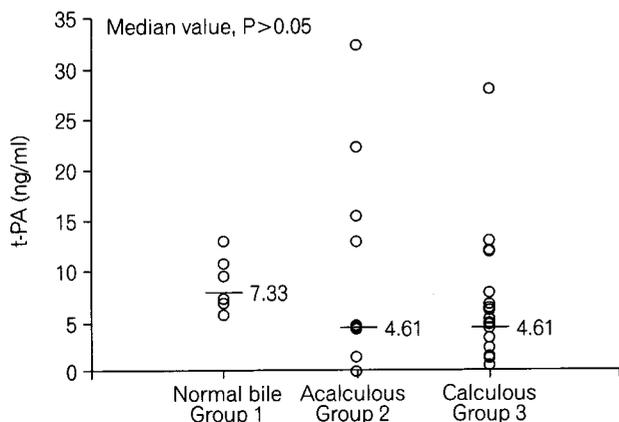


Fig. 1. Concentration of t-PA in bile.

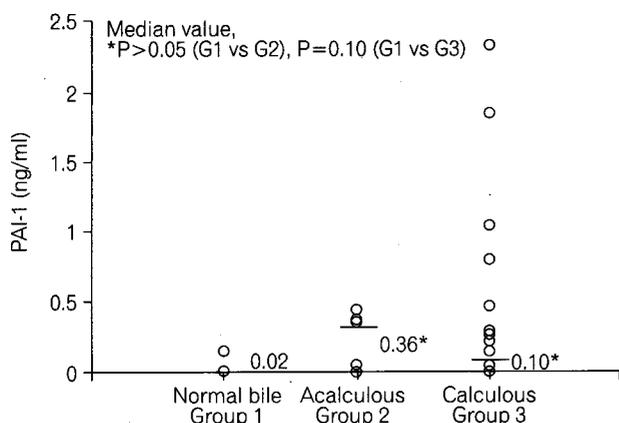


Fig. 2. Concentration of PAI-1 in bile.

duct obstructions and choledocholithiasis with cholangitis (acalculous-infected bile (group 2), median 0.36 ng/ml and calculous-infected bile (group 3), 0.1 ng/ml versus normal bile (group 1), 0.02 ng/ml,  $p < 0.05$ ), while there was no statistical difference in the choledochal bile concentration of plasminogen activator inhibitor-1 between the acalculous-infected (group 2) and calculous-infected choledochal bile (group 3) (Fig. 2).

## DISCUSSION

Tissue plasminogen activator constitutes an important agent in the fibrinolytic pathway and the levels are thought to have a major effect on fibrinolytic potential.<sup>4</sup> The physiological role of tissue plasminogen activator is to activate plasminogen to

plasmin, which in turn degrades fibrin to soluble degradation products. Fibrinolysis appears to be regulated by a specific molecular interaction between tissue plasminogen activator and fibrin, as well as between plasmin and the specific plasmin inhibitors.<sup>4</sup> Plasminogen activator inhibitor-1 is the primary inhibitor of tissue plasminogen activator, a key enzyme in fibrinolysis. An increased level of plasminogen activator inhibitor-1 is associated with impaired fibrinolytic function. Elevated levels of plasminogen activator inhibitor-1 have been observed in thrombolytic disease, acute myocardial infarction and deep vein thrombosis, as well as normal pregnancy and sepsis.<sup>5-9</sup>

Ever since plasminogen-activating activity was detected in human gallbladder mucosa and bile, it has been supposed that the gallbladder possesses a mechanism to prevent the accumulation of fibrin,<sup>3</sup> as it has been accepted that urokinase isolated from human urine possesses the same fibrinolytic activity in the urinary tract,<sup>10</sup> and that peritoneum also possesses a significant amount of fibrinolytic activity. Since reduced plasminogen-activating activity was a possible mechanism of adhesion formation,<sup>11</sup> and since the plasminogen activator inhibitor-1 was detected in higher concentrations in gallbladder bile in patients with gall stones, this increased level of plasminogen activator inhibitor-1 may play a part in gall stone formation by inhibiting fibrinolytic activity of plasminogen activator in the gallbladder bile.<sup>3</sup> We were interested in determining whether the human bile duct has similar properties which could have a role in the prevention of gall stone formation. There have been a few studies investigating the fibrinolytic properties of bile.<sup>1,3,12</sup> To date, the most consistent studies have arisen from Oshiba et al.,<sup>12</sup> who purified a plasminogen activator from human bile in 1969 and termed it bilokinase. Bilokinase has subsequently been characterized and found to be immunologically distinct from urokinase.<sup>13,14</sup> It is probable that tissue plasminogen activator and bilokinase are the same.

Our studies have confirmed the presence of tissue plasminogen activator and plasminogen activator inhibitor-1 in human choledochal bile also. Further studies are needed to localize the production of tissue plasminogen activator and plasminogen activator inhibitor in human choledochal bile. The concentration of tissue plasminogen activator in the choledochal bile was no different among the three groups (acalculous-

infected bile, median 4.61 ng/ml and calculous-infected bile, 4.61 ng/ml, versus normal bile, 7.33 ng/ml). However, we did not assay the plasminogen-activating activity, a functional measure of fibrinolytic activity, in human bile duct bile, so we were unable to analyze the relation between the tissue plasminogen activator and the plasminogen-activating activity. Plasminogen activator inhibitor-1 was present in choledochal bile in significantly greater concentrations in patients with acalculous cholangitis due to bile duct obstructions and choledocholithiasis with cholangitis compared with normal bile (acalculous-infected bile, median 0.36 ng/ml and calculous-infected bile, 0.1 ng/ml, versus normal bile, 0.02 ng/ml,  $p < 0.05$ ), but the bile concentration of plasminogen activator inhibitor-1 was no different between the acalculous-infected and calculous-infected choledochal bile. The plasminogen activator inhibitor-1 was found in greater concentrations in the choledochal bile from patients with infected bile compared with normal bile, but it was no different with the presence of bile duct stones. Bacterial inflammation is associated with a reduction in fibrinolytic activity.<sup>11,15</sup> These increased levels of plasminogen activator inhibitor-1 may be an epiphenomenon of infection of bile ducts, and do not seem to play a part in gall stone formation in bile ducts. The pathogenesis of bile duct stone formation is complex and includes the supersaturation of bile, nucleation of crystals, infection, and bile stasis.<sup>16</sup> The role of bacterial contamination of the bile and fibrinolysis requires further research. To determine whether the presence of bacterial contamination in the stone group biased the results, we need to analyze the bile of the stone group without bacterial contamination. Further studies are required to investigate the nucleation properties of the fibrinolytic components of choledochal bile to determine whether there is a relation between disturbances in bile fibrinolysis and bile duct stone genesis.

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