Fibrinolysis and the biliary tree

D M Scott-Coombes, S A Whawell, E G Havranek, J N Thompson

Abstract

Aims—To investigate the fibrinolytic activity of normal and calculous human bile.

Methods—Fibrinolytic properties of the biliary tract were studied in patients with gall bladder stones (n=7) compared with acalculous gall bladders (n=8).

Results—Bile plasminogen activating activity was detected in a wide range in both groups (calculous bile median 0.35 IU/ml; range: 0.06–6.59, versus normal bile 0.70 IU/ml; 0.19–3.56). There was no difference in the bile concentration of tissue plasminogen activator between the two groups (calculous bile median 21.5 ng/ml versus normal bile 9.5 ng/ml), which was present in much greater concentrations than urokinase (calculous bile median 0.10 ng/ml versus normal bile 0.36 ng/ml). Both plasminogen activators were detected in low concentrations in gall bladder mucosa. Plasminogen activator inhibitors-1 and 2 were detected in bile in significantly greater concentrations in patients with gall bladder stones (plasminogen activator inhibitor-1: calculous bile median 15 ng/ml versus normal bile <2 ng/ml, plasminogen activator inhibitor-2: 157 ng/ml versus <6 ng/ml, $p < 0.05$).

Conclusions—Human bile possesses fibrinolytic activity and the principal plasminogen activator in bile seems to be tissue plasminogen activator. Plasminogen activator inhibitors were present in greater concentrations in stone bile and may be a factor in the pathogenesis of gall stone formation.

(Gut 1997; 40: 92–94)

Keywords: fibrinolysis, biliary tract, gall bladder stones.

Fibrinolytic activity has previously been detected in animal bile.^{1,2} We are interested in determining whether the human biliary tract has similar properties, which could have a role in the prevention of gall stone formation. The aim of this study was to investigate the presence of fibrinolytic proteins in gall bladder mucosa and bile and compare between normal and calculous bile.

Methods

Patients

Approval was obtained from the Hospital Ethical Committee, and all patients gave informed consent. Samples were obtained

from two groups of patients. Those undergoing elective surgery for symptomatic gall bladder stones and those undergoing elective laparotomy for non-inflammatory conditions without clinical, biochemical, radiological or operative evidence of gall bladder stones.

Sample technique

Bile was aspirated from the gall bladder from all patients upon entry into the peritoneal cavity, snap frozen in liquid nitrogen, and then transferred to a freezer at -20°C . In addition, biopsy specimens of gall bladder mucosa and peritoneum were sampled from patients with gall stones at completion of the cholecystectomy using a 6 mm diameter biopsy punch (Stiefel Laboratories, Wooburn Green, UK). The disc of tissue was wrapped in silver foil and snap frozen in liquid nitrogen before transfer to a freezer at -20°C .

Preparation of tissue homogenate

Gall bladder peritoneum and mucosa homogenates were prepared according to a previously described method.³ Briefly, biopsy specimens were thawed rapidly at room temperature, weighed, rinsed in buffer, and homogenised in buffer containing detergent (Triton X100; Sigma Chemicals, Poole, UK). The homogenate was centrifuged at 12 000 *g* for 20 minutes and aliquots of the supernatant stored at -20°C for no longer than four weeks before assay.

Assays

Tissue plasminogen activator, urokinase, and plasminogen activator inhibitor-1 and inhibitor-2 were measured by enzyme linked immunosorbent assay (Tintelize, Porton Cambridge Ltd, Maidenhead, UK). The lower limits of sensitivity for the assays were 1.5 ng/ml (tissue plasminogen activator), 0.1 ng/ml (urokinase), 2.0 ng/ml (plasminogen activator inhibitor-1), and 6.0 ng/ml (plasminogen activator inhibitor-2). Plasminogen activating activity was determined by the fibrin plate technique after incubation for 24 hours at 37°C and the results for bile expressed as IU tissue plasminogen activator/ml bile (lower limit of detection 0.005 IU/ml), and for gall bladder mucosa/peritoneum IU/cm² (lower limit of detection 0.02 IU/cm²).

Gall stone content was measured spectrophotometrically (620 nm) after fragmentation, dissolution of 10–15 mg samples (ethanol, ether, and chloroform), filtration, and dehydration (sulphuric and glacial acetic acid). Gall stones were classified according to the percent-

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Accepted for publication
29 July 1996

age cholesterol content; >70%: cholesterol, 15–70%: mixed and <15%: pigment.

Bile samples were subjected to biochemical analysis according to established techniques. Biliary bile acid and cholesterol concentrations were determined using enzymatic spectrophotometric methods^{4,5} and biliary phospholipid concentrations were determined from the measurement of formed inorganic phosphate.

Statistics

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

Results

Eight patients (five female; median age 48, (range 29–83)) underwent elective cholecystectomy for symptomatic cholelithiasis, but one patient was excluded as the aspirated bile was white. For the remaining seven patients, the gall bladders were filled with bile at surgery, and all samples had a total bile lipid content in excess of 5 g/dl (median 6.2, range 5.1–8.8). The gall stones were either mixed cholesterol/pigment variety ($n=4$) or cholesterol stones ($n=3$). Bacteriological analysis of the bile identified *Coliform* species in two of these specimens. Six gall bladders were found to have chronic cholecystitis on histology, while in one patient a diagnosis of acute on chronic cholecystitis was made. Eight patients (six female; 72 (42–81)) underwent elective laparotomy for non-inflammatory conditions (five colectomies, three gastrectomies).

Plasminogen activating activity was detected in gall bladder mucosa, in which both tissue plasminogen activator and urokinase were measured in low concentrations (Fig 1). Neither plasminogen activator inhibitor-1 or 2 were detected in gall bladder mucosa. There was no significant differences in the bile acid, phospholipid or cholesterol concentrations in the bile between the two groups. A wide range of plasminogen activating activity was measured in the bile from both groups of patients (Fig 2). While there was no statistical difference in the bile concentration of plasminogen activators between the groups,

Figure 2: Plasminogen activating activity (PAA) of bile sampled from patients with and without gall bladder stones. Bars represent median values.

tissue plasminogen activator was present in much greater concentrations compared with urokinase (Fig 3). Both plasminogen activator inhibitor-1 and plasminogen activator inhibitor-2 were detected in higher concentrations in gall bladder bile in the group of patients with gall stones ($p < 0.05$) (Fig 4).

Discussion

Ever since urokinase was isolated from human urine in 1951,⁶ it has been accepted that the urinary tract possesses a mechanism to prevent the accumulation of fibrin. We are interested in determining whether the human biliary tract has similar properties which could have a role in the prevention of gall stone formation. There have been few studies investigating the fibrinolytic properties of bile and most have reached conflicting conclusions, possibly as a result of varied techniques used to measure fibrinolytic activity.^{1,7,8} To date, the most consistent studies have arisen from Oshiba *et al* who purified a plasminogen activator from human bile in 1969 and termed it bilokinase.⁷ Bilokinase has subsequently been characterised and found to be immunologically distinct from urokinase.^{9,10}

Our studies have confirmed the presence of plasminogen activating activity, a functional measure of fibrinolytic activity, in human gall bladder bile and the total bile lipid content suggests that the gall bladders were functioning. Plasminogen activating activity was also present in gall bladder mucosa, although in lesser amounts compared with peritoneum (gall bladder serosa) – a membrane acknowledged to possess a significant amount of activity.¹¹ Tissue plasminogen activator was present in much greater concentrations in the bile compared with urokinase, and it is probable that tissue plasminogen activator and bilokinase are the same. One potential source of production of tissue plasminogen activator is the gall bladder mucosa, where tissue plasminogen activator was measured in similar concentrations to the peritoneum. Further studies are

Figure 1: Plasminogen activating activity (PAA), tissue plasminogen activator (t-PA), and urokinase (uPA) in gall bladder mucosa and serosa sampled from patients with gall bladder stones. Bars represent median values.

Figure 3: Concentration of tissue plasminogen activator (t-PA) and urokinase (uPA) measured in bile sampled from patients with and without gall bladder stones. Bars represent median values.

Figure 4: Concentrations of plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2) in bile from patients with and without gall stones. Bars represent median values ($p < 0.05$).

needed to localise production of bile tissue plasminogen activator.

This is the first study to report the presence of plasminogen activator inhibitors in human bile, and these were found in greater concentrations in the bile from patients with gall

bladder stones. These increased levels of plasminogen activator inhibitors may be an epiphenomenon or these proteins may play a part in gall stone formation. Bacterial inflammation is associated with a reduction in peritoneal fibrinolytic activity.^{3 11} To determine whether the presence of bacterial contamination in the stone group biased the results, we re-analysed the data excluding these two patients and found that the difference between the two groups remained. The role of bacterial contamination of the bile and fibrinolysis requires further research. The pathogenesis of gall stone formation is complex and includes the supersaturation of bile, nucleation of cholesterol crystals, and gall bladder hypomotility.¹² Further studies are required to investigate the nucleation properties of the individual fibrinolytic components to determine whether there is a relation between disturbances in bile fibrinolysis, biliary biochemical properties, and gall stone genesis.

- 1 Zahorska-Markiewicz B, Starzewski J. The fibrinolytic activity in bile. *Acta Med Pol* 1972; 12: 199-204.
- 2 Oshiba S, Ariga T, Sawai H, Imai H, Endo E. On a plasminogen activator in mammalian bile: extraction method, species differences and enzymatic properties. *Proc Int Union Physiol Sci* 1977; 13: 572-5.
- 3 Thompson JN, Paterson-Brown S, Harbourne T, Whawell SA, Kalodiki E, Dudley HAF. Reduced human peritoneal plasminogen activating activity: possible mechanism of adhesion formation. *Br J Surg* 1989; 76: 383-4.
- 4 Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; 20: 470-5.
- 5 Mashige F, Imai K, Osuga T. A simple and sensitive assay of total serum bile acids. *Clin Chim Acta* 1976; 70: 79-86.
- 6 Williams JRB. The fibrinolytic activity of urine. *Br J Exp Biol* 1951; 32: 530-7.
- 7 Oshiba S, Hata S, Okamoto S. A plasminogen activator in mammalian bile. *Jpn J Physiol* 1969; 19: 212-9.
- 8 Benedetti-Valentini S, Luminari M. Esiste en potese fibrinolitico nella bile? *Min Med* 1957; 48: 2462.
- 9 Oshiba S, Ariga T. Purification and characterization of biliary plasminogen activator bilokinase. *J Biol Chem* 1983; 258: 622-8.
- 10 Ariga T, Oshiba S, Seki T, Imai H, Sawai H. Studies on bilokinase, a biliary plasminogen activator: immunologic property and organ distribution. *Thromb Res* 1989; 56: 37-48.
- 11 Vipond MN, Whawell SA, Thompson JN, Dudley HAF. Peritoneal fibrinolytic activity and intra-abdominal adhesion formation. *Lancet* 1990; 335: 1120-2.
- 12 Schoenfield LJ, Carey MC, Marks JW, Thistle JL. Gallstones: an update. *Am J Gastroenterol* 1989; 84: 999-1001.