Clinical significance of plasma endothelin levels in patients with biliary atresia

Abstract  Biliary atresia (BA) is the end-result of a destructive inflammatory process that affects intra- and extrahepatic bile ducts, leading to fibrosis and obliteration of the biliary tracts with the development of biliary cirrhosis and portal hypertension (PH). Endothelins (ET) are 21-amino-acid peptides of endothelial origin with potent vasoconstrictor activity that bind to various cells of the liver. Nothing is presently known about plasma ET levels in BA. The aim of this study was to determine the clinical significance of plasma ET levels in patients with BA after hepatic portoenterostomy (Kasai’s procedure) and to correlate these with liver function tests (LFT) and PH. We measured plasma concentrations of ET in 19 patients with BA (5 boys and 14 girls; mean age 11.6 ± 5.5 years) after portoenterostomy and 10 age-matched controls. Patients were grouped according to outcome based on LFT: group A consisted of 9 patients with an “unfavorable outcome” and Group B 10 patients with a “favorable outcome”. The plasma ET levels were measured using a highly sensitive and specific enzyme immunometric assay (EIA). No patient had ascites or hepatorenal syndrome. Plasma ET levels were significantly higher in patients with BA than in controls (3.42 ± 0.42 vs. 1.75 ± 0.39 pg/ml, respectively; P < 0.01) and in patients in group A than in group B. (3.75 ± 0.25 vs. 3.06 ± 0.23 pg/ml, respectively; P < 0.01). In group A, plasma ET levels were higher in patients with PH (n = 4) than in those without PH (n = 5) (3.99 ± 0.06 vs. 3.64 ± 0.22 pg/ml, respectively; P < 0.05). We conclude that plasma ET levels are high in patients with BA, especially those with severe biliary cirrhosis, and that ET may partially contribute to development of PH in BA. The results of the present study also suggest that plasma ET concentrations may be a useful marker in the follow-up of patients with BA.

Key words  Endothelin  ·  Biliary atresia  ·  Portal hypertension

Introduction

Biliary atresia (BA) is the end-result of a destructive inflammatory process that affects intra- and extrahepatic bile ducts, leading to fibrosis and development of biliary cirrhosis and portal hypertension (PH) [9]. Increased vascular resistance is one of the main causes of the clinical manifestations of PH (ascites, splenomegaly, esophageal varices, and increased porto-systemic collateralization).

Endothelins (ET) are 21-amino-acid peptides of endothelial origin with the most potent vasoconstrictive activity described to date [16]. Three isoforms (ET-1, ET-2, and ET-3) have been described. Although ET was originally identified as a potent vasoconstrictor, it is now known to have a wide variety of biological activities on vascular as well as nonvascular tissues [16]. Increased plasma ET concentrations have been described in various diseases such as pulmonary hypertension, arteriosclerosis, renal failure, migraine, and vascular disease [16]. Although there have been recent reports on plasma ET concentrations in patients with cirrhosis [1, 12], virtually nothing is known about plasma ET levels in BA.

The aim of this study was to determine the clinical significance of plasma ET levels in patients with BA following hepatic portoenterostomy (HPE, Kasai’s procedure) and to correlate these with the degree of hepatic dysfunction as assessed by liver function tests and the presence or absence of PH.

Materials and methods

Nineteen patients who had undergone HPE for BA (5 boys and 14 girls; mean age 11.6 ± 5.5 years) and 10 healthy children (4 boys...
and 6 girls; mean age 10.5 ± 5.3 years) were studied after obtaining informed consent. None of the patients had ascites or hepatorenal syndrome. They were divided into an “unfavorable outcome” group (group A) and a “favorable outcome” group (group B). Group A (n = 9) patients were jaundiced (total bilirubin > 1.5 mg/dl) and had raised serum levels of glutamic oxaloacetic transaminase (＞40 IU/l), glutamic pyruvic transaminase (＞35 IU/l), and gamma-glutamyl transpeptidase (＞55 IU/l). Four of these patients also had PH. Group B patients (n = 10) were anicteric and had normal serum levels of the above enzymes. None of the patients in group B had PH.

Peripheral blood samples (1.5 ml) were collected in sterile EDTA tubes on ice and centrifuged within 1 h. Supernatants were stored at -70°C. The serum liver enzymes were measured by routine laboratory methods. Plasma ET levels were measured using commercially available enzyme-linked immunosorbent assay kits (Cayman Chemical, Michigan) and expressed in pg/ml plasma. The minimal detectable concentration is ≥1.5 pg/ml after a 2-h development period and ≥3.0 pg/ml after a 30-min development period. Comparisons of ET levels between BA patients and controls and between patients with and without PH were performed using Student’s t-test.

**Results**

Plasma ET levels were significantly higher in patients with BA than in controls (3.42 ± 0.42 vs 1.75 ± 0.39 pg/ml, respectively; P < 0.01) and were also significantly higher in patients in group A than in group B (3.75 ± 0.25 vs 3.06 ± 0.23 pg/ml, respectively; P < 0.01) (Fig. 1). In the patients with unfavorable outcome (group A), plasma ET levels were higher in patients with PH (n = 4) than in those without PH (n = 5) (3.99 ± 0.06 vs 3.64 ± 0.22 pg/ml, respectively; P < 0.05) (Fig. 2).

**Discussion**

Endothelins (ET) are produced by epithelial, mesangial, neuronal and glial, and liver cells [7, 8]. They have been shown to have powerful mitogenic effects on fibroblasts and smooth-muscle cells [6]. The formation and release of ET is stimulated by hypoxia and ischemia as well as numerous other factors such as angiotensin II, thrombin, and transforming growth factor beta 1. ET has also been shown to stimulate the secretion of nitric oxide, arginine, vasopressin, and atrial natriuretic peptide [3]. Recent studies have shown that in the liver, ETs act predominantly on the lipocytes (Ito cells). ET-induced contraction of Ito cells has been shown to be a prominent feature of liver injury [10]. It has also been suggested that in more advanced fibrosis, ET may participate in contraction of collagen bands, leading to parenchymal distortion [4]. As such, there appears to be evidence to suggest that ET levels should accurately reflect ongoing hepatic fibrosis.

The prognosis of patients with BA has improved remarkably since the introduction of Kasai’s operation [9]. However persistence of intrahepatic pericholangitis in spite of successful relief of obstruction is known to be the cause of progressive hepatic fibrosis and PH in a majority of these patients [9]. In this study, plasma ET levels were significantly higher in BA patients who had objective evidence of persistent and progressive liver damage (group A) following successful HPE compared to patients in the group with favorable outcome (group B). Plasma ET levels in BA patients with a favorable outcome were also significantly higher than in controls, thus implicating ET in the mechanism of liver injury in patients with BA.

It is of interest that the highest levels of ET were seen in patients with PH. Recent studies have also shown a definite link between plasma ET levels and portal pressure in patients with cirrhosis, suggesting that ET may contribute to the development and perpetuation of PH [2, 11]. This view is further supported by the results of several animal and human studies in which administration of ET induced vasoconstriction and increased portal pressure [5, 13–15].

In conclusion, the results obtained from the present study demonstrate that elevation of ET may be harmful to liver function in patients with BA, and that plasma ET concentrations may be a useful clinical indicator in