Biliary Atresia

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Biliary atresia is a neonatal obstructive cholangiopathy characterized by a fibrosclerosing obliteration of the extrahepatic bile duct that uniquely presents in the first months of life (1). The condition occurs in approximately 1 in 8,000 to 1 in 15,000 live births and accounts for 30% of all cases of cholestasis in young infants. Biliary atresia is the most frequent cause of chronic end-stage liver disease in children and the leading indication for liver transplantation in the pediatric population, accounting for 40% to 50% of all pediatric liver transplants. Two forms of this disease have been recognized recently. In the *embryonic* type, which occurs in 15% to 30% of cases, the cholestatic manifestations of biliary atresia present at birth in association with other extrahepatic anomalies, including polysplenia, portal vein anomalies, malrotation, abdominal situs inversus, and congenital heart disease (2). With the classic *perinatal* type of biliary atresia, accounting for 70% to 85% of cases, the clinical features of jaundice and acholic stools manifest within the first 2 weeks of life with no other associated abnormalities. With both subtypes, complete obstruction of bile flow develops as a result of a sclerosing fibro-obliteration of the extrahepatic bile duct.

No curative therapy for biliary atresia exists. The initial treatment is surgical, involving resection of the obliterated extrahepatic bile duct and creation of a hepatoportoenterostomy (Kasai procedure). The Kasai procedure should be performed before 2 months of age to successfully reestablish bile flow. For many infants, delayed recognition of the disease and delayed referral for specialty care remain major obstacles to optimal timing of this initial surgical intervention. Yet even with early surgery, most patients (70–80%) eventually develop end-stage biliary cirrhosis and require liver transplantation (3). In the United States, the annual cost for this disease is approximately $65 million. Despite current treatment efforts, biliary atresia remains the most serious and costly liver disease that affects infants.

The cause and pathogenesis of either embryonic or perinatal biliary atresia are not known. Genetic, viral, and host immune factors are putative etiopathogenic mechanisms of this disorder. The lack of suitable animal models hampers this research. Furthermore, the paucity of cases at any one center has limited the availability of sufficient human tissue samples to study pathogenic mechanisms and has limited the development of amply sized clinical trials to test new treatment strategies. Research efforts should be directed toward defining disease cause and pathogenesis, developing methods of early detection, and creating more effective therapeutic interventions. More formalized educational programs in the medical and lay communities are necessary to allow earlier recognition of this condition.

AREAS OF EMPHASIS

Genetic Factors

Although several reports describe families in which male and female siblings have the disease, identical twins discordant for the condition have been described frequently. Generally, biliary atresia is not considered to be an inherited disease. However, with the less common embryonic-type biliary atresia, which is associated with other congenital malformations, genetic mutations that result in defective morphogenesis may be important to disease pathogenesis. Anomalous development of bile ducts with progressive cholestasis, situs inversus, and cystic kidneys has been described in the transgenic *inv* mouse, raising the possibility that alterations in the inversin gene may be one genetic mechanism for some cases of embryonic biliary atresia (4). With perinatal biliary atresia, a significant increase in the HLA B12 antigen and haplotypes A9-B5 and A28-B35 has been reported in one study but not confirmed in another (4–6). Thus genetic factors may play a role in the susceptibility to develop biliary atresia, and this area must be explored further. The two subtypes, although phenotypically the same with respect to the bile duct lesion, may have different genetic predispositions and etiopathogeneses.

Viral Agents

In the landmark article by Landing (7) on the pathogenesis of neonatal obstructive cholangiopathies including biliary atresia, a virus was proposed as the most plausible triggering agent for the disease. In a viral animal model of extrahepatic bile duct injury, infection of 3-week-old weanling mice with reovirus type 3 causes a fibrosclerosing lesion of the murine extrahepatic bile duct similar to the lesion in neonatal biliary atresia.

Along with this finding, the investigators found evidence for serologic reactivity to reovirus 3 in several children with biliary atresia. However, subsequent studies using an immunoperoxidase technique and polymerase chain reaction (PCR) were unable to detect reovirus 3 in affected human hepatobiliary tissue (8). A recent study using tissues frozen at surgery showed an association of reovirus RNA with the presence of biliary atresia (9). Riepenhoff-Talty et al. (10) and Petersen et al (11) reported the development of extrahepatic biliary atresia in newborn mice inoculated orally with group A rotavirus. The Petersen group found that prophylactic treatment with interferon alpha before rotavirus A infection in this experimental model prevented the hepatobiliary obstructive lesion. Although Riepenhoff-Talty et al. (12) reported rotavirus group C in some patients with biliary atresia. Bobo et al. (13), using a more sensitive nonisotopic reverse transcriptase PCR enzyme immunoassay, found no evidence of rotaviral group A, B, or C RNA in human tissue samples from patients with biliary atresia. Sporadic case reports of cytomegalovirus, papillomavirus, or Epstein-Barr virus infection in biliary atresia also have appeared, but no consistent findings have been reported. To date, no specific virus has been identified definitively as the cause for either the embryonic or perinatal subtype of biliary atresia.

Host Immune Factors

Several studies have examined the role of host immune factors in the pathogenesis of biliary atresia. Dillon et al. (14), examining liver biopsy tissue from patients with biliary atresia, found aberrant expression of the MHC class I molecules, ICAM-1, but not MHC class II determinants in intrahepatic bile ductular epithelial cells. In contrast, Broom (15) noted increased surface expression of HLA-DR antigen in addition to ICAM-1 on intrahepatic bile ductular epithelial cells in liver biopsy samples from patients with biliary atresia. These authors also found increased CD4-positive T cells, predominantly in the portal tracts. Kobayashi et al. (16) suggested that the overexpression of hepatocyte MHC class II antigen (HLA-DR) and CD68 (macrophage-associated) antigen may indicate a poor prognosis in these patients. In concert with these findings, Davenport et al. (17) found that low expression of CD68 within the liver and biliary remnants is associated with better postoperative prognosis. Schreiber et al. (18) have developed a murine model of extrahepatic bile duct immune injury to characterize the immunopathogenic processes that may be involved in the pathogenesis of biliary atresia. They have proposed a two-hit phenomenon for the pathogenesis of the bile duct injury in biliary atresia, one that depends on immunogenetic vulnerability to environmental precipitating factors, such as a toxin or a viral agent (19). Further studies are necessary to determine immunologic factors that may mediate disease progression.

Clinical Diagnostic and Prognostic Indicators

The clinical presentation and biochemical features of biliary atresia overlap those of neonatal hepatitis and other causes of newborn cholestasis. Developing more sensitive and specific tests for early diagnosis of affected patients is a major challenge. An early and reliable diagnostic screening test for biliary atresia would overcome the problem of late referral. Moreover, early diagnosis may allow the introduction of new and timely therapeutic interventions that could improve long-term outcome. Currently, the most reliable test for diagnosing biliary atresia, aside from exploratory laparotomy, is percutaneous liver biopsy. A specimen containing 5 to 7 portal tracts can be 93% accurate for the diagnosis of this disease. No serologic tests or imaging studies are diagnostic for the condition. Hepatobiliary scintigraphy has been used to assess neonatal cholestasis; however, failure of excretion may be seen in both biliary atresia and neonatal hepatitis. The sensitivity and specificity of scintigraphy, with concomitant administration of phenobarbital, for diagnosing biliary atresia is about 95% and 93%, respectively. The finding of a triangular cord at the porta hepatis may be a useful ultrasonographic sign of biliary atresia (20). Endoscopic retrograde cholangio pancreatography (ERCP) may be of diagnostic value. However, the use of this procedure is relatively new for this age group, is invasive and technically challenging in young infants, and is not readily available. Magnetic resonance cholangiography may become an important tool for diagnosing biliary atresia, but further studies are required (21). Although decreased amniotic GGTP levels may occur in animal models of cholestasis, currently no prenatal screening exists for either the embryonic or perinatal type of extrahepatic biliary atresia. The costs and benefits of screening all newborn infants for increased serum direct bilirubin concentration remain to be determined (22). One study has shown that newborn screening for biliary atresia using tandem mass spectrometry to measure serum bile acids is not feasible (23).

The timing of the Kasai procedure will predict the efficacy of the surgery. Patients with perinatal biliary atresia who undergo surgery before 60 days of age have a high likelihood of obtaining good initial bile flow (80–90%) and improved liver function. In contrast, in more than 80% of patients who undergo surgery after 90 days of age, jaundice will not be cleared (3). Patients in whom portoenterostomy has failed usually will require liver transplant within 1 year. A recent French national study reported an overall 10-year survival rate of 68% for patients with biliary atresia who were managed at a time when both the Kasai procedure and liver transplantation were readily available treatment options (24). Portoenterostomy outcomes in patients with embryonal biliary atresia are significantly worse, regardless of the timing of the surgery. Patients with this subtype are more likely to require early liver transplantation. The surgical experi-

ence of the treatment center is an important prognostic factor for overall outcome (24,25). Repeated episodes of bacterial cholangitis after portoenterostomy also is associated with progressive fibrosis of the intrahepatic biliary tree and worse prognosis. Variceal hemorrhage in patients with biliary atresia and increased serum bilirubin concentration portends a poor prognosis (26). A hepatic artery resistance index greater than 1 measured by ultrasound Doppler predicted rapid deterioration in children with biliary atresia after portoenterostomy (27). Histologic predictors of poor outcome after a portoenterostomy include the presence of cirrhosis in initial liver biopsy tissue and nonpatent bile ducts or absence of ducts at the level of the liver hilus. Azarow et al. (28) analyzed the liver biopsy findings of 31 patients with biliary atresia and concluded that the presence of syncytial giant cells, lobular inflammation, focal necrosis, bridging necrosis, and cholangitis was associated with failure of the portoenterostomy, whereas bile in zone 1 was associated with clinical success.

Treatment Strategies

A variety of treatment strategies must be considered in the future management of biliary atresia. Although the Kasai surgery remains the initial procedure of choice for infants, adjuvant therapeutic agents coupled with the Kasai surgery must be evaluated. For example, postoperative steroid therapy may improve operative outcome (29). The role of prophylactic antibiotics or ursodeoxycholic acid require further study. Other strategies including the use of antivirals, anticytokines, antifibrogenic, and cytoprotective agents also may prove beneficial.

RESEARCH GOALS

The specific research areas and goals are as follows:

1. Genetic factors: Researchers must identify and define genetic factors that may be responsible for pathogenesis of embryonic or perinatal biliary atresia.
2. Viral agents: Although a specific viral infection has not been identified as the cause of biliary atresia, continued research in this area is required. Use of animal models to delineate the mechanisms of virally induced bile duct injury is necessary.
3. Host immune factors: The nature of the immunologic processes that lead to fibrosclerosing bile duct injury in biliary atresia requires further study. Possible maternal immune factors must be explored. Moreover, the cellular and molecular biology of the progressive fibrosis in the extrahepatic duct and the liver, and the factors responsible for the development of biliary cirrhosis in biliary atresia, must be defined.
4. Progressive injury: The role of toxic bile acids, oxidant stress, adenosine triphosphate depletion, endo-

toxins and bacterial cell wall antigens, activation or recruitment of inflammatory mediators, activation of degradative hydrolases, and mitochondrial damage in the pathogenesis of end-stage liver disease must be examined.
5. Clinical diagnostic and prognostic disease indicators: Developing diagnostic markers for the disease is necessary to identify patients early in infancy for optimal treatment strategies. More precise predictors of outcome for the various treatment options are required. A cost–benefit analysis of universal postnatal screening of serum direct bilirubin or bile acid levels should be undertaken to examine its effect on the economics, health, and quality of life of affected infants.
6. Treatment strategies: Treatment strategies to decrease bile duct destruction, ongoing intrahepatic injury, and progressive fibrosis must be explored in animal models and eventually in infants with biliary atresia. Combined with portoenterostomy surgery, medical treatments may decrease, delay, or prevent ongoing intrahepatic bile duct destruction and fibrosis.

RESEARCH STRATEGIES

Genetic Factors

Individual genotypes for each phenotypic subtype of biliary atresia must be identified. Studies are necessary to better define the immunogenetics of perinatal biliary atresia. Because embryonic biliary atresia is associated with other congenital malformations, defects in the genetic regulation of morphogenesis deserve further study. A transgenic mouse with an insertional mutation in the proximal region of mouse chromosome 4 (*inv* mouse) develops situs inversus and jaundice (4). Similar models that investigate the factors regulating normal development of the bile duct are necessary to help advance our understanding of disease pathogenesis. A search for mutations of human homologues of murine genes that determine the laterality of embryonic biliary atresia must be performed. The embryologic origin and development of the intrahepatic biliary system has been examined, using immunostaining with cytokeratin antibodies specific for biliary epithelial cells (30). The embryonic ductal plate develops from primitive hepatic precursor cells contiguous to portal vein mesenchyme; it eventually differentiates into single-layer intrahepatic bile ductular cells to form the intrahepatic biliary tree. Further characterization of the regulation of mesenchymal epithelial cell–cell interaction in the embryogenesis of the intrahepatic biliary ductal system is required. Parallel studies of the development of the extrahepatic biliary system, which derives from a different site in the embryonic foregut, are necessary.

Viral Agents

Using novel molecular biology techniques, ongoing search for specific viral triggering agents for either sub-

type of biliary atresia is warranted. Animal models of virally induced biliary atresia, including the rotavirus A and reovirus 3 models, require further study to characterize disease pathogenesis (8–13). Moreover, these models should be used to define in vivo mechanisms of immune recognition and cytotoxicity of virally infected bile duct epithelial cells. In addition, we must use these models to develop new diagnostic tests for bile duct injury and to test novel treatment strategies for this disease.

Host Immune Factors

We must understand the role of the immune system in mediating the progressive fibrosclerosing lesion of the extrahepatic bile duct in biliary atresia. Studies that identify the phenotype of the extrahepatic bile duct cellular infiltrate and cytokines, and that examine the determinants of immune recognition of bile duct epithelial cells and of disease susceptibility, must be ongoing (14–17,31). For these studies, ample human tissue from patients with biliary atresia must be available. Parallel studies should be conducted in vivo using existing animal models of either virally or immunologically induced bile duct injury (11,18). In vitro studies using human or animal bile duct epithelia in cell culture should be used to specifically characterize mechanisms of epithelial cell–immunocyte interaction (32). The cellular and molecular events that mediate bile duct fibrosis during infancy must be clarified. Moreover, the factors responsible for the extrahepatic and intrahepatic progression to end-stage biliary cirrhosis require study. How hepatic stellate cells are activated to produce collagen and the role of the bile duct cell in triggering this fibrotic process also require investigation.

Progressive Injury

Infants with biliary atresia are cholestatic, and concentrations of bile acids are increased. Hydrophobic bile acids are hepatotoxic and may contribute to end-stage liver disease in these patients through a variety of mechanisms, including mitochondrial injury, oxidant stress, adenosine triphosphate depletion, and activation of degradative hydrolases (33). Bacterial infection of the biliary tree (cholangitis) is associated with ongoing destruction of intrahepatic bile ducts. In turn, some of these events may stimulate fibrosis. The role of bile acids and bacterial cell-wall products, and their interactions with hepatocytes, Kupffer cells, and stellate cells in the mediation of progressive liver disease in biliary atresia require further study.

Diagnostic and Prognostic Disease Indicators

We must establish a central registry for biliary atresia patient information to collect epidemiologic data, to gen-

erate portoenterostomy outcome statistics, to initiate multicenter clinical therapeutic trials, to collect and provide human tissues for basic science research, and to serve as an educational resource for the professional and lay communities. We must develop strategies for early diagnosis, which may include instituting a neonatal screening program. We must better identify which infants require portoenterostomy as initial treatment and which should directly undergo liver transplantation. We must develop novel pharmacologic, antimicrobial, and nutritional treatments to optimize the outcome of portoenterostomy. There is an ongoing need to develop strategies for the economic use of donor organs, such as split grafts and living-related donors. The development of extracorporeal liver assist devices and research in xenotransplantation will have significant impact on this patient population.

Treatment Strategies

The possible impact of novel treatment strategies to augment recovery from the portoenterostomy procedure must be explored. Pharmacologic interruption of the mechanistic processes by which the liver and intrahepatic bile ducts are injured after portoenterostomy are of high priority. Examples would include preventing bacterial cholangitis, stimulating bile flow, cytoprotection of the hepatocytes and the bile duct epithelium, decreasing oxidant stress, inhibiting or blocking stimulators of fibrogenesis, and decreasing immune-mediated bile duct injury. Any intervention in infants with biliary atresia will require a multicenter trial to recruit adequate numbers of patients.

PROJECTED TIMETABLE AND FUNDING REQUIREMENTS

If efforts are appropriately funded and focused, many of these research goals may be accomplished during the next 5 to 7 years. A multi-institutional grant should be awarded by the National Institutes for Health to establish a consortium of 10 to 15 pediatric hepatology investigative centers that care for large numbers of infants with biliary atresia and other neonatal diseases. This consortium would collect epidemiologic data in a uniform fashion; prospectively obtain and store serum, leukocyte DNA, and tissue specimens from patients and appropriate control groups (and family members when indicated); develop hypotheses; and participate in investigation of the etiopathogenesis of biliary atresia and in interventional trials. A commitment for the first 5-year period of $5 million to $10 million would be necessary to achieve these goals. In addition, five new RO1 grants of approximately $150,000 to $200,000 each, to support direct costs, should be awarded to individual investigators to

study the basic mechanisms that underlie the pathogenesis of embryonic and perinatal biliary atresia.

HEALTH AND ECONOMIC OUTCOMES

The actual costs for biliary atresia per annum in the United States are difficult to calculate. If the Kasai procedure is used for initial management, the cost of hospitalization and surgical care of these infants through the first year of life has been estimated at $17,500 to $20,000 (data presented at NDDAB symposium, September 1994). With an estimated incidence of 1 in 10,000 live births, approximately 410 infants are treated yearly in the United States for an annual cost of $7.6 million. Between the years 1990 and 1994, more than 1000 patients with biliary atresia received transplants. Assuming 260 transplantations per year at an average cost of $225,000, the expenditure of transplant care for these patients is $58.5 million per annum. Other costs not included in this calculation are as follow:

1. The cost of medical care after the Kasai procedure and until liver transplantation (on the average, a 4- to 6-year period) (Many patients need urgent medical management of complications, which include recurrent ascending cholangitis, malnutrition, gastrointestinal variceal bleeding, ascites, hypersplenism, and infection.)
2. The cost of a second Kasai procedure, which some patients require.
3. The cost of early second transplantation, which about 10% of transplant recipients require.
4. The cost of drugs to support a child with end-stage liver disease or to prevent rejection after transplant.
5. The economic impact of this disease on the patient's immediate family in terms of missed workdays.
6. The immeasurable emotional costs on the patient and extended family.

CONCLUSION

Biliary atresia remains the most common reason for liver transplantation in infants and children. Biliary atresia has enormous emotional and financial consequences for children, parents, healthcare providers, and third-party payers. It is also a disorder that offers major opportunities for advancing our understanding of the biology of bile duct growth and its interaction with the immune system. With appropriate funding to advance research in this area, patients with biliary atresia and other progressive sclerosing cholangiopathies ultimately may avoid many of the often severe medical consequences of these diseases, and ultimately the need for liver transplantation may be diminished.

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