Development of New Therapeutic Strategies for Inborn Errors of Metabolism Involving the Liver

Alex P. Mowat

King’s College School of Medicine and Dentistry, Department of Child Health, Variety Club Children’s Hospital, Bessemer Road, London SE5 9PJ, United Kingdom

In only a small percentage of genetic disorders or inborn errors of metabolism can the clinical features be ameliorated by manipulating the metabolic pathway by dietary measures, drugs, vitamins, cofactor supplementation, enzyme induction, or end-product replacement (1). Even if such intervention has dramatically beneficial effects in the short term, the long-term outcome may be less than ideal; for example, the 40% incidence of developmental delay and 80% incidence of ovarian failure in patients with galactosemia treated with a galactose-free diet (2). The development of improved modes of treatment applicable to a much wider range of disorders is imperative.

In view of the key role of the liver in metabolism, much attention has been directed toward the functioning of this organ. Brief consideration is necessary of some microanatomical and physiological features which are required for effective hepatic function. A major task of the liver is the uptake of substrates, exogenous (absorbed from the intestine) and endogenous (the metabolic products of various organs) (3). These are metabolized, stored, and distributed as required to other organs. The liver in health is able to maintain in the hepatic vein blood a mixture of solutes and macromolecules within finely regulated concentration limits, adjusted both for the metabolic demands of the body at that time and for recent dietary intake. These functions are modulated by regulatory factors which are mainly humoral. They induce great variation in the phenotypic expression of the organ. The liver has a unique structure that facilitates these tasks. It is worth emphasizing that there are at least three types of cells involved in maintaining metabolic homeostasis. The prime cell is, of course, the hepatocyte, with over 5,000 identified metabolic pathways expressed within each cell. Hepatocytes show considerable functional heterogeneity. Those near the portal tract frequently express very different metabolic activity from those near the portal vein.

A second unique cell is the specialized endothelial cell which lines the sinusoid.
separating the circulating blood from the space of Disse. These cells have, in their cytoplasm, fenestrae occurring in small patches, called sieve plates, which provide channels of variable diameter. Thus solutes and macromolecules can pass readily from the plasma space to the space of Disse, into which abut the microvilli on the hepatocyte cell surface. Partially metabolized chylomicrons can pass through these channels. The other function of the sinusoidal cell is receptor-mediated uptake of a wide range of macromolecules.

The third cell is another specialized endothelial cell, the Kupffer cell, which has a unique surface membrane which facilitates adherence, pinocytosis, and phagocytosis. Like other macrophages, these cells are engaged in antigen processing and antigen presentation. It has become increasingly clear that these three types of cells interact physiologically and pathophysiologically in important ways. Kupffer cells, for example, can be stimulated to release a factor which decreases cytochrome P450 activity in hepatocytes. They can also be stimulated to produce a factor that inhibits protein synthesis within the hepatocyte. Substances taken up by Kupffer cells or endothelial cells may subsequently be transferred to hepatocytes.

The fourth cell, the fat storage cell or Ito cell, may also influence hepatic function by producing extracellular matrix components (EMCs). These may have a direct effect on hepatocyte function by interacting with specific receptors (e.g., for laminin or fibronectin) on the hepatocyte surface. The metabolism of these cells is influenced by the other liver cells and by cytokines from migratory cells of the reticuloendothelial system. In chronic liver disease the rate of synthesis exceeds that of degradation of EMCs which accumulate and impair circulation and cell-to-cell transfer. In bone marrow transplantation the replacement of host Kupffer cells by that of the donor may have an adverse effect on intracellular interactions and may stimulate formation of ECM.

With respect to treatment in the infant, it should be remembered that at birth hepatocyte plates are usually two cells thick. It is not until the age of 5 that the plates are one cell thick, maximizing contact between blood and hepatocytes. Hepatic function is, of course, dependent on parenchymal blood flow. It depends on the gradient between the pressure in the terminal portal vein, estimated at 50 ml H2O, and in the initial hepatic vein branch, approximately 10 ml H2O. It should be appreciated that the biotransformation systems which are most active and best studied in the liver may be present in other organs, such as the kidney, small intestine, and endocrine organs.

There are at present four ways of modifying hepatocyte metabolism in genetic disorders which require consideration. The first exploits the techniques of genetic engineering to introduce a new function into the hepatocyte. In this Chapter (this volume), Dr. Friedman considers the role of hepatotropic viruses as targeting agents to introduce new DNA into the host cell. Another approach has been to use specific receptors such as that for transferrin or asialoglycoproteins, which are found only on hepatocytes. Material bound to these hepatocyte receptors are internalized. Thus if a genetic message is combined with asialoglycoprotein in a plasmid carrier system which includes the required gene and its promoter, and agents that allow binding
and integration of DNA in a nondamaging reaction, gene expression is possible. In rats injected intravenously with such a targeted DNA complex, it has been possible to obtain expression of the bacterial enzyme, chloramphenicol acetyl transferase in the hepatocytes. To date, gene expression has not been shown to persist beyond 14 days (4,5).

Another means of replacing deficient enzymatic activity is hepatocyte replacement. Although this has been achieved in the short term in experimental animals (6), major problems remain, including the prevention of the rejection and the provision of hepatotrophic factors which promote hepatocyte engraftment, functioning, and endogenous regeneration. A further obstacle is the identification of a source of hepatocytes and development of means of storage and transportation. In vitro hepatocytes are very dependent on the culture matrix (7). In vivo they survive, on histological assessment, for 6 months in the pancreas and for 9 months in the spleen. A demonstration of functional survival has been of shorter duration. Detectable albumin synthesis in analbuminemic rats has been achieved (8).

A further and possibly more promising approach has been to utilize auxiliary (heterotopic) liver transplantation in which the patient’s liver remains in situ, but another liver or part of a liver is placed surgically within the abdomen. Recently, surgeons in Rotterdam have reported encouraging short-term results using this mode of transplantation in patients with advanced chronic liver disease (9). Whether a heterotopic liver would survive if a structurally intact host liver remains in situ without the provision of hepatotrophic factors (poorly understood) is a matter for speculation.

Orthotopic liver transplantation is thus now the only established mode of therapy that may offer a good quality of life for children with metabolic disorders that cause liver damage or in whom a deficient metabolic process in the liver causes damage to other vital organs, such as the brain or kidneys (10–12). For some disorders orthotopic liver transplantation will suffice. Others will require transplantation of other organs, such as the kidneys, pancreas, or lung.

THE PRESENT STATUS OF ORTHOTOPIC LIVER TRANSPLANTATION

Liver transplantation, first successfully performed in a child of 18 months in 1968, is now established as a therapeutic option offering long-term survival with a good quality of life in almost all forms of chronic liver disease and fulminant hepatic failure (10,13,14). Liver transplantation remains a formidable surgical procedure. The recipient is likely to have one or more life-threatening complications in the operative or immediately postoperative period. Primary graft failure, vascular thrombosis, and the prevention of rejection without causing toxicity such as renal damage or increasing susceptibility to infection are major problems (15). Cytomegalovirus infection is strongly associated with chronic rejection, with disappearance of bile ducts (vanishing bile duct syndrome) (16).

Most difficulties occur in the first months after transplantation, resulting in a 1-year graft survival that is usually less than 60%. Supply of donors of suitable size
and blood group remains a major limiting factor in developing liver transplantation in children. Two recent developments have improved donor availability. Children can be successfully engrafted using part of a liver from a much larger donor, with results comparable to those obtained with whole organ grafting (17,18). Extended in vitro preservation of the liver using a solution developed in the University of Wisconsin has important logistic implications but may also allow time for prospective cross-matching between the donor and the recipient (19). An important limitation with respect to metabolic disease is the finding that donor Kupffer and endothelial cells are replaced by recipient cells early in the post-transplantation period (20). Thus liver transplantation is ineffective for disorders affecting primarily Kupffer cells, such as Niemann-Pick type C (white blood cell sphingomyelinase activity normal) (21).

Starzl and co-workers, reporting their experience in the first 1,000 liver transplants performed since cyclosporin became available as an immunosuppressant, recorded a 1-year actuarial survival of 74%, with a 5-year survival of 64% (10). It should be noted, however, that the 5-year survival of children under 1 year of age, the age at which some of the most severe metabolic disorders would benefit from transplantation, was only 37%, significantly lower than that of older children or adults. Long-term problems following liver transplantation include chronic rejection, opportunistic infection, lymphoproliferative disease associated with Epstein-Barr virus infection, bile duct stenosis, both anastomotic and nonanastomotic, and the development of malignancy. A recent estimate of cost of liver transplantation, with an average hospital stay of 41 days, was greater than $140,000. Long-term costs are likely to remain high, with cyclosporin costing approximately $5,000 per year (14).

Patients who avoid major complications have a good quality of life with catch-up growth and normal development (22). The longest survivor to date remains well 17 years after transplant. A more enthusiastic attitude toward donor procurement by pediatricians and other health care professionals will be necessary to make sufficient livers available to fully utilize the potential of this technique unless legislative changes are made to facilitate donor retrieval (23).

Note: For the discussion, see the next chapter.

REFERENCES


**DISCUSSION**

*Note: The discussion relative to this chapter is to be found at the end of the chapter that follows.*