

Experimental Models of Hepatic Fibrosis in the Rat

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Abstract

The rat is a frequently used experimental model in studies involving human disease. We review several methods of inducing hepatic fibrosis and cirrhosis in rats. These include induction by hepatotoxins and hepatocarcinogens such as carbon tetrachloride, dimethylnitrosamine, thioacetamide and furan; the hepatotoxin-cum-nutrient, alcohol; a high fat-low choline-low protein diet; immunologic agents such as heterologous serum or bacterial cell wall products; and obstructive jaundice and biliary cirrhosis by common bile duct ligation.

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Introduction

Hepatic fibrosis and cirrhosis of the liver are complex diseases which, in man, may be caused by such varied factors as alcohol, hepatitis, schistosomiasis, biliary atresia, malnutrition and exposure to carcinogens and hepatotoxins. Although hepatic fibrosis is characterised by the massive deposition of extracellular matrix components in the liver which causes a distinctive pathology, there are also differences which depend on the aetiological factor causing the disease.

There has not been, to date, an animal model that completely mimics the spectrum of disease symptoms seen in man. The ideal animal model should: (a) have similar morphologic features to the human disease being studied; (b) have a gradual and discrete progression of change in the liver pathology; (c) be highly reproducible with a low mortality rate; (d) be reversible or irreversible, depending on the nature of the study and (e) develop pathophysiologic sequelae.¹ Although a "perfect" model for the study of hepatic fibrosis and cirrhosis of all aetiologies has not been developed, significant contributions have been made to the understanding of the cellular and molecular mechanisms underlying hepatic fibrosis and cirrhosis, and to the efficacy of various therapeutic agents, using the rat as an experimental model.

The experimental models of hepatic fibrosis and cirrhosis in the rat can be categorized by the aetiological factor, namely, (1) hepatotoxins, (2) nutritional, (3) immunologic and (4) biliary.

Hepatotoxin-induced Hepatic Fibrosis

Carbon Tetrachloride

One of the oldest and most widely used hepatotoxins in experimentally-induced hepatic fibrosis is carbon tetrachloride (CCl₄). It has been used in a wide variety of laboratory animals and has been administered either by gastric gavage, subcutaneous injection or inhalation (reviewed by Tsukamoto et al¹). Carbon tetrachloride is activated by oxidases in the liver to yield the trichloromethyl (CCl₃) free radical. This reactive free radical then either abstracts a hydrogen atom from unsaturated membrane lipids to initiate lipid peroxidation, or reacts with the sulphhydryl groups of proteins. The hepatotoxicity of CCl₄ is potentiated by chemicals that induce cytochrome P450 isozymes, such as phenobarbitone, acetone and ethanol.¹

A highly reproducible method for developing severe decompensated micronodular cirrhosis and fibrosis which mimics, both histologically and systemically, alcoholic cirrhosis in humans is the chronic CCl₄/rat model developed by Proctor and Chatamra.² The hepatotoxin is administered in such a way that there is a repetitive infliction of damage to the liver which is confined between a reversible "hepatitis" and death from acute liver failure. Male Wistar rats with starting weights of 200 g were pretreated with 35 mg/dl phenobarbitone in the drinking water for 10 to 14 days. After the initial pretreatment period, when the rats were about 250 g, they were treated with CCl₄ for 8 to 10 weeks. The hepatotoxin was administered by gastric gavage, at 7-day

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intervals. The dose of toxin used was individually tailored to the weight of each rat and the optimum dose was 0.04 ml CCl_4 /250 g rat.

With 8 to 10 weeks of treatment with CCl_4 , a shrunken and finely nodular liver with a histologic picture of micronodular cirrhosis that was not immediately reversed when CCl_4 was stopped was produced.² However, the fibrosis may diminish after a prolonged discontinuation of the toxin. There was fatty metamorphosis and centrilobular necrosis, septal and nonseptal fibrosis, and fully developed cirrhosis, resembling the pattern seen in alcoholic liver injury in man.¹ When CCl_4 is given intragastrically, it enters the liver via the portal vein and is selectively concentrated by the rat liver in a ratio of 13:1 with respect to the blood. The intragastric route is thus more efficient at delivering the hepatotoxin to the liver, compared to inhalation of CCl_4 vapour or subcutaneous/intraperitoneal injection.²

We have found the Proctor and Chatamra method of inducing hepatic fibrosis in male Wistar rats a very convenient and reproducible model. It can be established with relative ease in a short time period and does not require specialised equipment or skills. The main concern, however, is to ensure that the CCl_4 does not aspirate into the lungs of the animal.

Hepatocarcinogens

The two most commonly used carcinogens in experimentally-induced liver fibrosis are dimethylnitrosamine (DMNA)³ and thioacetamide (TAA).⁴ While chronic low doses of these compounds are carcinogenic, high doses result in necrosis in the liver and subsequent fibrosis. Thrice-a-week intraperitoneal injections of DMNA in the rat induces necrosis of the centrilobular and periportal areas within 1 to 2 weeks which is replaced by fibrosis after 4 weeks, followed by formation of central-portal septa and cirrhosis after 13 weeks. Unlike CCl_4 -induced hepatic fibrosis, DMNA does not cause fatty change in hepatocytes and the fibrosis and cirrhosis is stable several months after discontinuation of the drug.^{1,3}

Repeated administration of 300 mg/l TAA in drinking water to rats induces fibrosis in 2 to 3 months and micronodular cirrhosis after 3 months at an incidence rate of 100% with a 20% mortality rate. There is fibrous enlargement of the portal tracts, formation of portal-portal or portal-central septa and cholangiocellular proliferation. The fibrotic lesions persist for two months after withdrawal of TAA.^{1,4} The DMNA and TAA models of hepatic fibrosis may be useful in studies involving liver cancer associated fibrosis and cirrhosis in man.

Furan is a cholangiocarcinogenic agent that induces fibrosis in young adult male Fisher 344 rats.⁵ The rats weighing 160 to 190 g were gavage-fed 45 mg/kg body weight of furan in corn oil daily for up to 32 days. The right and caudate liver lobes had cholangiofibrotic le-

sions with numerous metaplastic intestinal glands and hyperplastic bile ductules in a dense fibrotic stroma, while the histopathology of the left lateral and median liver lobes ranged from mild fibrosis to biliary cirrhosis. The differences in the susceptibility of the rat liver lobes were postulated to be due either to the streaming of furan in the portal venous circulation, resulting in a higher concentration of the carcinogen being routed to the right and caudate liver lobes, or to interhepatic lobe differences in the metabolism of furan. The hyperplastic bile ductules present in furan-treated rat livers are similar to those obtained with bile duct ligation of the liver (see later section).

Nutritionally-induced Hepatic Fibrosis

Alcohol

The excessive intake of alcohol is a major aetiological factor in hepatic fibrosis and cirrhosis in man. Due to the aversion of most animals to ethanol, and the inability to control the nutritional state and maintain a high blood alcohol level at the same time, there were initially problems establishing an appropriate animal model for alcohol-induced hepatic fibrosis. Both of these issues are addressed in the Tsukamoto-French rat model, which has been found to be a suitable model for alcoholic liver disease. Chronically implanted gastric catheters were used to feed male adult Wistar rats with a diet in which 47% of the calories were contributed by ethanol. Sustained blood alcohol levels were achieved for 2 to 12 weeks in rats that were also on a controlled fat diet. In a low dietary fat regimen, there was intense fatty infiltration and focal centrilobular necrosis. An increase in the intake of corn oil potentiated the effect of alcohol. In a high fat diet, there was advanced liver injury and liver fibrosis. With this model, it was possible to have a complete and independent control of the nutrient uptake via the implanted gastric catheter. There was a distinctive and reproducible progression of alcoholic liver injury from fatty liver, centrilobular necrosis and inflammation, to focal pericellular and perivenular fibrosis and focal bridging fibrosis, all within several months.

Another agent that potentiates the effect of alcohol is iron.⁶ While a intragastric infusion of 0.25% wt/vol carbonyl iron had no effect on liver histology or function of control male Wistar rats, the iron supplementation in rats fed with 49% of their total calories as ethanol exacerbated hepatocyte damage, promoted liver fibrogenesis and produced liver cirrhosis.⁶

High fat-low choline-low protein Diet

Liver cirrhosis was produced in rats that were on diets that were low in protein and choline and high in fat (reviewed by Tsukamoto et al¹). Within 6 weeks of such a diet there was steatosis or fatty degeneration in the

centrilobular zone due to choline deficiency and in the portal region due to a deficiency of some amino acids. In the following 6 to 12 weeks, there was formation of fibrosis from the central vein to adjacent central veins or portal tracts. After 12 to 24 weeks of feeding, cirrhosis occurred. Although the pathology resembled that seen in alcoholic liver disease, the variable response between animals, prolonged experiment duration, difficulty in studying the pathologic stages, and species differences in the susceptibility to choline deficiency limit the use of this model in experimental studies of hepatic fibrosis.

Immunologically-induced Hepatic Fibrosis

Heterologous Serum

Male Sprague-Dawley rats developed hepatic fibrosis when sensitised by three intraperitoneal injections with 4 mg human albumin (mixed 2:3 with Freund's incomplete adjuvant) at 10-day intervals and then injected twice a week for 5 to 11 weeks through the tail vein with 6 mg albumin. The immunisations led to deposition of antigen-antibody complex along the endothelial walls which triggered a tissue response, namely, fibrosis, in 75% of the rats. The fibrotic livers showed a total alteration of lobular architecture with thin fibrous bands partitioning the liver into lobules and bridging fibrosis linking portal spaces or portal spaces with central veins. There was some hepatocyte trapping, bile duct hyperplasia and tissue eosinophilia. However, there was little necrosis and no Kupffer cell hyperplasia or inflammatory reaction.⁷ This model would therefore facilitate in the assessment of the efficacy of therapeutic agents by allowing a distinction between pure antifibrotic and anti-inflammatory, radical scavenging or membrane protecting agents.

Bacterial Cell Wall Products

A single intraperitoneal injection of the peptidoglycan-polysaccharide fragment of group A streptococcal cell wall produced chronic inflammatory lesions associated with the formation of fibrotic tissue in the livers of euthymic female LEW/N rats but not in athymic *rnu/rnu* athymic rats.⁸ This T lymphocyte-dependent liver fibrosis would be a useful model for the investigation of the cellular and molecular mechanisms involving macrophages, T cells and the cytokines released from them.

Biliary Cirrhosis

Obstructive jaundice and experimental biliary cirrhosis by ligation-of the common bile duct has been conducted with variable success in rats, as well as other animals.⁹ The main technical problems include reversibility of the histologic changes due to recanalization of the obstructed ductal system, bile accumulation in the distensible extrahepatic biliary passages when there is low common bile duct ligation, and a high mortality.

Through midline laparotomy from the xiphoid to the pubis, the bile ducts of anaesthetized rats were isolated, doubly ligated, and divided. In the sham operated animals, the bile duct was manipulated without ligation and division. The fascia and skin were then closed.⁹ A retrograde injection of 1.5 ml/kg body weight of a mixture of prolamine/ethanole (Ethibloc[®]) following double ligation of the choledochal duct has also been used to obtain biliary fibrosis in rats.¹⁰ The retrograde injection of Ethibloc was supposed to induce complete cirrhosis and reproducible portal fibrosis in the rats. There is also a lack of significant histological inflammation and necrosis in this model. As with the albumin-induced immunologic model, this model would be useful in studies of potential antifibrotic agents.

Summary

Just as human hepatic fibrosis is varied in its aetiology, so is there no one specific experimental model that manifests all the characteristics of hepatic fibrosis. Nevertheless, each of the models reviewed here facilitates the understanding of the cellular and molecular mechanisms involved in the process and provide tools for the assessment of the efficacy of various antifibrotic agents.

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