

Liver Fibrosis and Cirrhosis

Introduction

Liver fibrosis results from chronic injury such as viral hepatitis (especially hepatitis B and C), alcohol abuse, drugs, metabolic diseases due to overload of iron or copper, congenital abnormalities or autoimmune attack of hepatocytes or bile duct epithelium. It is characterized by the encapsulation or replacement of injured tissue by a collagenous scar. Liver fibrosis is reversible, whereas cirrhosis, the later stage of fibrosis progression, featuring regenerative nodules surrounded by fibrous bands, is generally irreversible. Liver cirrhosis leads to an impaired liver function with an increased intrahepatic resistance (portal hypertension) and the development of hepatocellular carcinoma (HCC).

Carbon tetrachloride (CCl₄)-induced hepatic fibrosis and cirrhosis in rodents is a well established and widely accepted experimental model for the study of liver fibrosis and cirrhosis. In many aspects it mirrors the pattern of human disease associated with toxic damage. For example, the α -SMA expression, Stellate cell activation and key matrix components including collagen-1, MMPs and their inhibitors TIMPs have been indicated in the pathogenesis of this model. CCl₄ induction elicits a reproducible and predictable fibrotic response in liver, making it a valuable basis for preclinical pharmacology studies of anti-fibrotic and anti-cirrhotic therapeutics and the pathophysiology study of liver fibrosis-cirrhosis-HCC changes.



PharmaLegacy Models and Research Tools

Model	Acute liver injury & fibrogenesis model	Fibrosis and cirrhosis model					
Animal	BALB/c mice	Wistar rats					
Toxin	CCl ₄	CCl ₄				Bile duct ligation	
Disease time	Onset Day 1-3, with recovery	Self-limiting injury: 1 wk	Early fibrosis: 4wks	Reversible fibrosis: 6 wks	Early cirrhosis: 8 wks	Micronodular cirrhosis: 12 wks	Onset 1-10 weeks, no recovery
Similarities to human disease	Acute injury with significant hepatocellular necrosis	Chronic progression with fibrosis, cirrhosis, ascites and liver failure				Chronic progression with fibrosis, cirrhosis. Irreversible	
Characteristics	Reliable; Ideal model for studying the effects on acute liver injury and early fibrotic changes	The duration required for cirrhosis mainly depends upon the CCl ₄ dosage. Liver carcinogenesis may develop due to the carcinogenic effect of CCl ₄ as well as CCl ₄ -induced liver cirrhosis. Good for investigating hepatocarcinoma growth in the liver cirrhosis environment				Rapid progression to cirrhosis. Short term for cholangitic injury; long-term for fibrosis/cirrhosis	
Primary endpoints	Liver functions: Liver fibrogenic stress: Histopathology: Biomarkers: Ascites:	Serum ALT, AST, bilirubin, globulin and albumin Hepatic hydroxyproline Collagen by Sirius red staining, connective tissue by Masson's trichrome staining, SMA- α and collagen IHC Collagen I, collagen III, TGF- α , β by quantitative RT-PCR Incidence and volume					
Additional endpoints	Fibrosis biomarkers: Hepatocyte regeneration: Apoptosis:	MMP, TIMP, HGF, TNF- α , cyclin by quantitative RT-PCR, Western blot, or ELISA IHC for PCNA, Ki67, BrdU TUNEL					
Special endpoints	TBARS, Hepatic glutathione, MDA content, Cytochrome P450 2E1					Cyclooxygenase-2 (COX-2)	

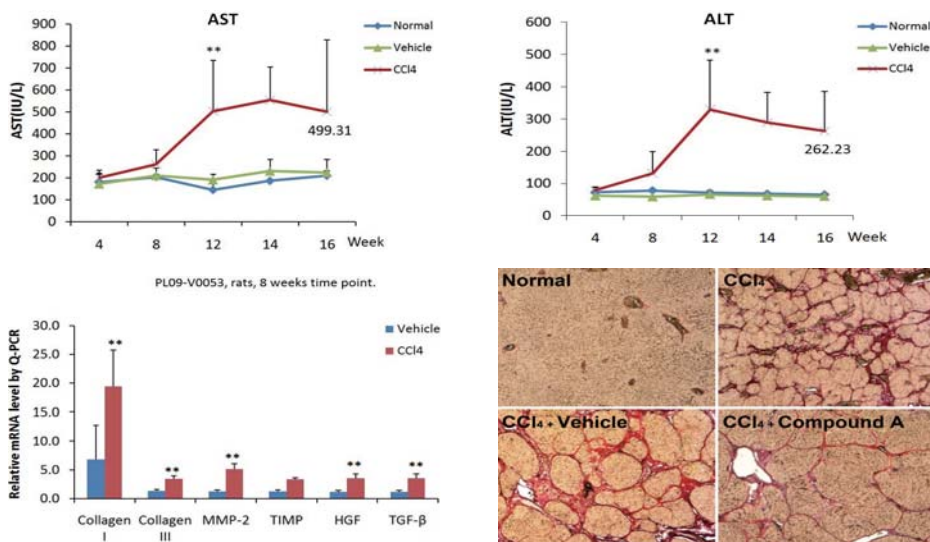
Related Capabilities

- * CCl₄-induced liver fibrosis and cirrhosis in mice (chronic)
- * Bile duct ligation induced liver fibrosis and cirrhosis in mice
- * Primary hepatic stellate cells culture system

Case Study - PLP Induced EAE Model

Liver fibrosis and cirrhosis induced by chronic injection of CCl₄ in Wistar rats

The AST and ALT in blood are increased with the duration of administration; a number of biomarker expressions are significantly increased in the liver at the end of 8 weeks; the hyperplastic nodules, surrounded by the fibrous bands, that are positively stained by Sirius Red are clearly formed in the liver at 16 weeks, but are ameliorated by Compound A treatment. (n=6, *P<0.05, **P<0.01 vs Vehicle by Bonferroni multi-comparison test)



PharmaLegacy Models and Research Tools

Efficacy studies for drug compounds/candidates:

- * Serum biochemical endpoints
- * Fibrotic grade evaluation in liver
- * Quantification of fibrotic tissue in liver (Sirius red)
- * Quantification of SMA-α positive cells
- * Incidence and volume of ascites

Molecular pharmacology for proof of concept studies:

- * Vitality of activated primary stellate cells
- * Morphological alterations of the hepatocytes
- * Hepatic hydroxyproline, hepatic/serum glutathione, malondialdehyde (MDA) content
- * Expression of cyclins, PCNA, Ki67 and BrdU for regeneration of hepatocytes

Mechanistic investigation:

- * Angiogenesis inhibitors
- * Angiotensin receptor antagonists
- * Vasoactive modulators
- * Hepatic vasculature analysis by immunostaining of anti-vWF, VEGF-A, angiopoietin-1, angiopoietin-2, placental growth factor
- * VCAM-1, ICAM-1 expression; CD11b, CD3, CD31 expression
- * Apoptosis of activated hepatic stellate cells

Bio-marker analysis of involved signaling pathways

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