



Mini Review

Liver Diseases from Lab to Clinic

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Abstract

Although, the great advance in experimental work in pharmacology, yet there is a gap between the different animal models and the real disease condition in human which affects the development of drugs. Here I will review a short comparison between the human pathology and the animal model pathology. I choose three of the most common benign hepatic conditions fatty liver, cirrhosis and hepatitis as they represent a high percentage of Egyptian patients at the out-patient clinic. Non Alcoholic Fatty Liver Disease (NAFLD) is a major growing health problem in our country. No animal model represents the whole clinical and metabolic feature of NAFLD although feeding the animals with special high fat diet represent some of these pathological feature but not all. Liver cirrhosis has many causes and shows a pleiotropic pathological feature. Chemical and mechanical methods for induction of cirrhosis are available but no single method can mimic the human pathology. Viral hepatitis is a unique cause of hepatitis that affects human and chimpanzee only. To simulate the pathological feature of viral hepatitis in small animals certain chemical methods are used to activate the immune response or by using genetically engineered rats which are too expensive.

Introduction

Liver diseases are most common health problems in our country and even in the Middle East. After 10 years working on the pharmacotherapy of the liver diseases, I think that there is still gap between the lab research and the management of patients at clinics. In this review, I will try to summarize the different rat models of liver diseases, the common pathways that most therapeutic approaches working on and the application of these approaches on the patients based on the recent valid international publications.

The Most Common Type of Benign Hepatic Lesions that are Induced in our Lab

Fatty liver disease

Based on recent epidemiological studies, fatty liver disease is the 2nd most common cause of liver cirrhosis in Egypt and even in the

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world. Fatty liver disease is of two types: Alcoholic Fatty Liver and Non Alcoholic Fatty Liver Disease (NAFLD).

Alcoholic Liver Disease (ALD)

The pathological feature of ALD in human include; steatosis begins in zone 3 (perivenular/centrilobular) which can be classified into microvesicular (small-droplet) or macrovesicular (large-droplet) types. Inflammation in Alcoholic Steato Hepatitis (ASH) is typically neutrophil-rich which has been attributed to the increase of chemokines, such as IL-8 and IL-17, in both the serum and the liver parenchyma. Histologic cholestasis is more often seen in ASH than Non Alcoholic ASH (NASH) and can be a key feature when distinguishing between these entities. Alcoholic foamy degeneration, is an uncommon pattern of microvesicular steatosis, which has been described as classically centrilobular and at times diffuse. An iron stain (i.e., Prussian blue) reveals an increase in parenchymal iron in later stage of ALD, particularly within Kupffer cells. Alcohol increases iron absorption in the gut. Significant siderosis, including significant iron staining within hepatocytes, should prompt consideration of a concurrent process such as hereditary hemochromatosis. Clinically, the patient may present with acute hepatotoxicity and markedly elevated serum gamma-glutamyl transaminase levels, with or without elevation of the transaminases. The pathogenesis is related to mitochondrial dysfunction and an identical histologic pattern can be seen in Reye's syndrome, tetracycline toxicity and fatty liver of pregnancy [1].

According to Brandon-Warner et al., no inclusive rodent model has been developed to date that accurately reflects the complete human pathology of ALD [2]. Several explanations as to why rodents do not develop ALD parallel to the human disease have been suggested;

1. Rodents (generally) have a natural dislike to alcohol consumption, but will consume alcohol partially for its caloric value. However, unlike humans, consumption does not increase over time.
2. The rate of alcohol catabolism is up to 5 times faster in rodents than humans, and rodents will stop consuming alcohol when blood acetaldehyde levels increase. However, differences in alcohol catabolism between humans and rodents must also be considered within the context of higher basal metabolic rates in rodents (in general) as compared to humans.
3. Alcohol avoidance is most likely favored as a result of survival in which impaired neurological function from alcohol consumption would increase susceptibility to marauder attack.
4. Inflammatory and innate immune responses and how they are influenced by translocation of intestinal LPS, are major components of ALD that must be considered when evaluating rodent models, as these responses are markedly different between humans and rodents. For example, neutrophil infiltration is considered a critical aspect of human steatohepatitis and progressive injury during ALD compared to only 10–25% in mice, where lymphocytes comprise 75–90% of leukocytes.
5. Risk for developing ALD varies among individuals. While significant components of human ALD variability is attributed to diet, smoking, type of alcohol consumed and other known risk factors for liver disease, underlying genotype also plays a significant role.

6. Polymorphisms in genes for alcohol metabolism (ADH, ALDH and CYP2E1), methionine metabolism, oxidative stress (manganese superoxide dismutase) and immune response (TNF α) have been reported. Much like their human counterparts, rodents demonstrate similar, varying degrees of ALD susceptibility. Inter strain differences in ALD initiation and progression are reported for rats and mice and thus become an important consideration in selecting species and strains for alcohol studies.
7. Weight gain was notably different (Long Evans>Sprague Dawley>Fisher) and correlated to diminished hepatic function, including lipid metabolism and increased hepatic damage (steatosis, lymphocyte infiltration, apoptotic and necrotic cell death, and altered hepatic architecture).
8. Levels of adiponectin, a fat derived hormone reported as protective against alcoholic fatty liver injury were depressed by alcohol consumption in all strains except those identified as (relatively) resistant to alcohol induced liver damage. As with other studies of ALD, hepatic damage correlated with global changes in lipid synthesis pathways, increased endoplasmic reticulum stress and disruptions to glutathione and methionine metabolism

Administration of Alcohol in Drinking Water (A-DW) over the course of several hours, days or weeks has the advantage of being the simplest mode of alcohol feeding and mimics human behavior patterns of intermittent alcohol use and changes in dietary intake. The A-DW method involves rodents being gradually weaned onto increasing concentrations of ethanol (10-40% (v/v)) by supplementing (the only source of) available drinking water with increasing amounts of alcohol while allowing animals to feed on standard rodent chow diets ad libitum. Using this approach animals develop significant hepatic steatosis and inflammation but do not progress to bridging hepatic fibrosis cirrhosis.

The A-DW model has advantages and limitations. In addition to rats exhibiting a natural aversion to alcohol, they also have a (relatively) rapid metabolic rate (~4-5 fold higher than that of humans) that may prevent Blood Alcohol Content (BAC) from consistently reaching high enough levels to exert the levels of hepatic injury associated with ALD. Based on BAC measurements in mice maintained on an A-DW regime (10/20% alcohol (v/v), alternating daily for 8-weeks) it was observed that BAC in males of 55-70mg/dL, while females had BAC of 50-65mg/dL amounts considered "moderate consumption" in humans.

In most studies, A-DW alone is sufficient to initiate steatosis but in order to stimulate inflammatory and fibrotic changes, a secondary stressor, such as iron, LPS, high-fat diet or Diethylnitrosamine (DEN) (in drinking water or by intraperitoneal injection) must be included. Similarly, addition of increased polyunsaturated fats administered concomitant with A-DW more closely approximated human ALD.

NAFLD

Dowman et al., summarized the pathogenesis of NAFLD in human based on a '2-hit hypothesis' [3]. The 'first hit', hepatic triglyceride accumulation or steatosis, increases susceptibility of the liver to injury mediated by 'second hits', such as inflammatory cytokines/adipokines, mitochondrial dysfunction and oxidative stress, which in turn lead to steatohepatitis and/or fibrosis. However, there is increasing recognition of the role that Free Fatty Acids (FFA) play in directly promoting liver injury, which has led to modification of this theory. In obesity and IR there is an increased influx of FFA to the liver. These FFA

either undergo β -oxidation or esterified with glycerol to form triglycerides, leading to hepatic fat accumulation. There is substantial evidence that FFA can directly cause toxicity by increasing oxidative stress and by activation of inflammatory pathways, therefore hepatic triglyceride accumulation may be a protective mechanism by preventing the toxic effects of unesterified FFA. Additionally, a further component or 'third-hit' has been added to reflect inadequate hepatocyte proliferation. In the healthy liver, cell death stimulates replication of mature hepatocytes which replace the dead cells and reconstitute normal tissue function. In chronic liver injury, the development of fibrosis/cirrhosis is dependent on the efficacy of hepatocyte regeneration and therefore cell death with impaired proliferation of hepatocyte progenitors represents the proposed 'third hit' in NAFLD pathogenesis.

The functional role of adipocyte derived cytokines (adipokines), is well described. Leptin is a 16 kDa hormone produced mainly by mature adipocytes whose actions include the regulation of energy intake and expenditure, regulation of the immune system and promotion of inflammation and fibrogenesis. Higher leptin levels are observed in obese patients and those with NAFLD, which are commonly regarded as states of leptin resistance. It remains plausible that leptin may have a functional role to play in the pathogenesis of NAFLD. In contrast to leptin, adiponectin is anti inflammatory and increases insulin sensitivity and the administration of recombinant adiponectin improves hepatomegaly, as well as the biochemical and histological parameters of NAFLD.

Numerous animal models of NAFLD/NASH have been reported to date; however, no animal model completely reflects hepatic histopathology and pathophysiology of human NAFLD/NASH. I will focus on one of the dietary models of NAFLD as it is the most commonly used in our lab for their low cost.

Takahashi et al., reported a diet model of NAFLD by using an High Fat (HF) diet (71% of energy from fat, 11% from carbohydrates and 18% from proteins) [4]. Rats fed this diet ad libitum for 3 wk showed elevated plasma insulin levels reflecting insulin resistance. Rats fed the HF diet developed marked panlobular steatosis and the hepatic lipid concentrations of these rats were approximately twice those of control rats fed the standard diet (35% fat, 47% carbohydrates and 18% protein). Like human NASH, the rats fed the HF diet developed oxidative damage in the liver. When dietary consumption was restricted, steatosis and inflammation in the liver, oxidative stress and plasma insulin levels were decreased. Feeding of an HF emulsion to Sprague Dawley rats also induced changes closely resembling human NASH.

Intragastric overfeeding of mice with an HF diet up to 85% in excess of standard intake for 9 wk has been reported to replicate the histopathological and pathogenic features of NASH. Neutrophilic infiltration and perisinusoidal fibrosis reminiscent of human NASH were observed. The White Adipose Tissue (WAT) exhibited increased TNF- α and leptin expressions and reduced adiponectin expression.

An HF diet is widely used to cause hepatic steatosis and NASH in experimental animals. However, it seems that the HF diet model produces variable results with regard to the degree of steatosis, inflammation and fibrosis, and the results depend on rodent species and strain, the fat content in the diet, the composition of dietary fat and the duration of treatment. For example, Sprague Dawley rats appear susceptible to steatohepatitis development when fed an HF diet, and this is likely associated with their susceptibility to diet induced obesity. On the other hand, it was reported that long term high saturated fat feeding did not induce hepatic steatosis and NASH in Wistar rats.

Among the HF models, the histopathology and pathophysiology of the intragastric overfeeding method most resemble those of human NASH. However, this method is difficult to implement, because it requires specific equipment and expertise. The optimization of the composition of the HF diet to reliably cause NASH in animals by ad libitum administration warrants future investigation.

Cirrhosis

Zhou et al., summarized the pathogenesis of liver cirrhosis in human and the different animal models commonly used to induce liver cirrhosis in Lab [5].

Apoptosis of hepatocytes is a common event in liver injury and contributes to tissue inflammation, fibrogenesis, and development of cirrhosis. Steatohepatitis enhances Fas mediated hepatocyte apoptosis, which correlates with active Nuclear Factor (NF)- κ B and disease severity. Both HCV infection and ethanol consumption induce hepatocyte apoptosis in animal models and humans, and induction may be related to down regulation of Bcl-2 signaling. Chronic HCV infection can induce hepatocyte G1 arrest and impair hepatocellular function and limit hepatic regeneration. In CCl₄-induced liver injury, hepatocyte apoptosis is induced at the early phase, which is followed by constant proliferation and if it persists, liver cirrhosis ensues at a later stage. Hepatocytes are the major sources of Matrix Metalloproteinases (MMP-2, MMP-3 and MMP-13) and tissue inhibitors of matrix metalloproteinases (TIMP-1 and TIMP-2); all of which are involved in the pathogenesis of liver cirrhosis in CCl₄-induced liver cirrhosis in rats. In the last fibrotic stage or cirrhosis, hypoxic hepatocytes become a predominant source of TGF- β 1, further exacerbating hepatic fibrogenesis.

PDGF is the strongest mitogen to HSCs among all polypeptide growth factors. PDGF family has four members, PDGF-A, -B, -C and -D. PDGF and its receptors are markedly over expressed in fibrous tissues and its activity increases with the degree of liver fibrosis. A variety of factors such as viruses, chemicals or mechanical damage to hepatocytes can induce KCs to synthesize and release PDGF. Upon binding to its specific receptor on the membrane of HSCs, PDGF activates corresponding signal molecules and transcription factors, leading to the activation of its downstream target genes and activation of HSCs. PDGF has been shown to up regulate the expression of MMP-2, MMP-9 and TIMP-1, and inhibit the activity of collagenase, thereby reducing ECM degradation.

TGF- β is the strongest known inducer of fibrogenesis in hepatic fibrosis. TGF- β is mainly synthesized by HSCs/my fibroblasts, KCs, LSECs and hepatocytes in the liver. The TGF- β 1 family is composed of six members and among them, TGF- β 1 has been shown to play a key role in the initiation and maintenance of liver fibrosis. The expression level of TGF- β 1 is increased in fibrotic liver and reaches a maximum at cirrhosis. The pro fibrogenesis effect of TGF- β 1 is complicated, involving multiple aspects: the primary effect of TGF- β 1 is to stimulate activation of HSCs and the TGF- β 1 autocrine loop in activated HSCs is an important positive feedback to the progression of liver fibrosis. TGF- β 1 induces expression of the matrix producing genes and inhibits degradation of ECM by downregulating expression of MMPs and promoting TIMP, leading to excessive deposition of collagenous fibers and promoting the development of liver fibrosis. In addition, TGF- β 1 has been shown to inhibit DNA synthesis and induces apoptosis of hepatocytes. TGF- β 1 induced apoptosis is thought to be responsible for tissue loss and decrease in liver size seen in cirrhosis. Given the critical

role of TGF- β 1 in the pathogenesis of liver cirrhosis, specific blockade of TGF- β 1/Smad3 signaling has shown some therapeutic value for liver fibrosis.

TNF- α is mainly produced by monocyte, macrophage, HSCs and KCs. It has proinflammatory activities and cytotoxic effects in these cells. In the process of liver fibrosis, TNF- α plays an important role in the activation of HSCs and synthesis of ECM. TNF- α can reduce the spontaneous apoptosis of activated rat HSCs by up regulating the anti apoptotic factors NF- κ B, Bcl-XL and p21^{WAF1}, as well as downregulating the proapoptotic factor p53. However, the effects of TNF- α on HSCs and fibrosis are complicated and even paradoxical, as demonstrated by studies showing that TNF- α could induce apoptosis in HSCs. TNF- α has also been shown to exert antifibrogenic effect in rat HSCs by reducing glutathione and inhibiting pro-collagen α 1 expression.

Interferon (IFN) is a family of soluble extracellular signaling molecules. Leukocytes synthesize IFN- α and IFN- β in response to virus infection and T cells secrete IFN- γ upon stimulation with various antigens and mitogens. IFNs possess antiviral activity is well-recognized for their antiviral effects. Patients treated with IFNs exhibit a regression of liver fibrosis even if viral eradication is not achieved, indicating that IFN itself has antifibrotic activity via triggering the apoptosis of HSCs. IFN- β could inactivate HSCs and decrease their production of α -Smooth Muscle Actin (SMA) and collagen through inhibition of the TGF- β and PDGF pathways. Similarly, IFN- γ has been demonstrated to reduce ECM deposition *in vivo* by inhibiting HSC activation via TGF β 1/Smad3 signaling pathways. Treatment of rats with fibrosis by IFN- γ led to a reduced production and deposition of collagen, laminin, fibronectin and pro-collagen type I in liver. However, the effect of IFNs on fibrosis is not consistent, as demonstrated by a recent study showing that IFN- α and IFN- γ may exert opposite effects on apoptosis in HSCs. IFN- α was shown to elicit an antiapoptotic effect on activated HSCs, whereas IFN- γ was found to exert proapoptotic effect on HSCs by downregulating heat-shock protein 70.

ILs are a group of cytokines initially found to be expressed by leukocytes, but later they were shown to be produced by a wide variety of cells, such as CD4 T lymphocytes, monocytes, macrophages and endothelial cells. ILs have a complicated role in immune response, inflammation and liver fibrogenesis. KCs and SECs can rapidly produce ILs in response to liver tissue damage. IL-1 can directly activate HSCs and stimulate them to produce MMP-9, MMP-13 and TIMP-1, resulting in liver fibrogenesis. In contrast, IL-1-recept or-deficient mice are less likely to sustain liver damage and exhibit reduced susceptibility to develop fibrosis. Deficiency of IL-1 α or IL-1 β also makes the mice less susceptible to develop liver fibrosis in animal models of steatohepatitis. Similarly, IL-1 receptor antagonists were found to protect rats from developing liver fibrosis in response to dimethylnitrosamine and blocking IL-1 signaling could markedly attenuate alcohol-induced liver inflammation and steatosis. IL-1 β was reported to increase the inflammatory and pro-steatotic chemokine monocyte chemoattractant protein-1 in hepatocytes and augment Toll Like Receptor (TLR4) dependent upregulation of inflammatory signaling in macrophages.

Another profibrotic cytokine is IL-17, whose expression level increases with degree of liver fibrosis, indicating that IL-17 may be involved in disease progression and chronicity. Studies in mice have shown that IL-17 induces liver fibrosis through multiple mechanisms, including upregulation of TNF- α , TGF- β 1 and collagen 1 α , which is dependent on Signal Transducer and Activator of Transcription

(STAT)3 signaling pathway and promotion of myofibroblast like change of HSCs.

IL-6 is a pleiotropic cytokine involved in inflammatory pathways, hematopoiesis and immune regulation. IL-6 can attenuate apoptosis and promote regeneration of hepatocytes through NF- κ B signaling and the Ras-MAPK pathway. IL-6 reduces CCl₄ induced acute and chronic liver injury and fibrosis. Pretreatment of fibrotic liver with IL-6 improves hepatic microenvironment and primes it for mesenchymal stem cell transplantation, leading to improvement in liver injury after fibrosis. Meanwhile, increased blood level of IL-6 has been found in patients with NAFLD and IL-6 could induce insulin resistance and inflammation in the liver, suggesting that IL-6 may play a role in the development of NAFLD.

miRNAs represent a family of small noncoding RNAs controlling translation and transcription of many genes, which have recently emerged as post-transcriptional regulators. miRNAs play a key role in various hepatic pathologies, including hepatitis, cirrhosis and hepatoma. miRNAs may play pro and antifibrogenic roles, depending on cellular context and the nature of the stimuli. miR-21 has an important role in the pathogenesis and progression of hepatic fibrosis. miR-21 can downregulate TGF- β expression and suppress HSC activation. TGF- β 1 induces expression of miR-181a and miR-181b, and the latter can promote HSC proliferation by regulating p27 and the cell cycle. Elevation of serum level of miR-181b is suggested as a potential diagnostic biomarker for patients with cirrhosis. miR-214-5p can increase expression of fibrosis-related genes (such as MMP-2, MMP-9, α -SMA and TGF- β 1) in LX-2 cells and therefore, it may play crucial roles in HSC activation and progression of liver fibrosis. miR-221 and miR222 are upregulated in human liver in a fibrosis progression dependent manner and in mouse models of liver fibrosis. TGF- α or TNF- α induce expression of miR-222, which can bind to the CDKN1B (p27) 3'-Untranslated Region (UTR) and regulate expression of the corresponding protein. Other fibrosis associated miRNAs have been identified. For example, miR-199a, miR-199a*, miR-200a and miR-200b were positively and significantly correlated with progression of liver fibrosis in both mouse and human studies. Over expression of these miRNAs significantly increases the expression of fibrosis related genes in HSCs. miR-571 is upregulated in human hepatocytes and HSCs in response to TGF- β . miRNA-150 and miRNA-194 are reduced in HSCs isolated from experimental rats with liver fibrosis. It has been demonstrated that these two miRNAs inhibit HSC activation and ECM production, at least in part, via inhibition of c-myc and rac1 expression. In contrast, several miRNAs such as miR-29, miR 19b, miR-146a and miR-133a are markedly downregulated in HSCs isolated from experimental animals with liver fibrosis and restoration of these miRNAs attenuates hepatic fibrogenesis. It is thought that miRNAs can serve as biomarkers for HSC activation and liver fibrosis progression and may represent therapeutic targets for hepatic fibrosis and cirrhosis.

Many types of animal model have been developed in mice, rats, rabbits and pigs to mimic the complicated process of fibrosis and cirrhosis. Animal models of liver fibrosis and cirrhosis can be induced by either chemical compounds and toxins such as CCl₄, Thioacetamide (TAA) and dimethylnitrosamine. Special diet, such as choline deficient, L-amino acid-defined, methionine deficient diet and HFD. Animals develop NAFLD and cirrhosis when they are fed these diets alone or in combination with other chemical agents. Physical methods also commonly used; bile duct ligation creates obstruction of the extra hepatic bile duct, leading to cholestasis and subsequent injury to biliary epithelial cells and hepatocytes, infiltration of inflammatory cells

in the portal area, fibrous tissue proliferation and formation of liver fibrosis.

The human liver is a complex organ with cell-cell and cell-matrix interactions and extra hepatic crosstalk. This complexity causes the difficulty in presenting animal models able to simulate liver fibrosis as global entities with relevant metabolic and immunologic backgrounds and specific hepatic features.

Hepatitis C

The T-cell chemo-attractant, interferon-inducible protein 10 (IP-10) is strongly expressed in HCV-infected liver. Antigenic peptides derived from the cleavage of exogenous viral proteins are recognized by CD4+ T-helper cells in association with MHC class II molecules on the surface of APC. T-helper lymphocytes have an immunoregulatory function through the secretion of lymphokines that support either Cytotoxic T Lymphocyte (CTL) generation (the T-helper type 1 cytokine profile: IL-2, IFN- γ) or B-cell function and antibody production (the type 2 profile: IL-4, IL-5, IL-10) [6].

Human Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infect only chimpanzees and humans. Analysis of both viruses has long been hampered by the absence of a small animal model. The recent development of human hepatocyte chimeric mice has enabled us to carry out studies on viral replication and cellular changes induced by replication of human hepatitis viruses. Various therapeutic agents have also been tested using this model. In the present review, we summarize published studies using chimeric mice and discuss the merits and shortcomings of this model [7].

The intravenous injection of the plant lectin Concanavalin A (ConA) is a widely used model for acute immune-mediated hepatitis in mice. In contrast to several other models for acute hepatic damage, ConA induced injury is driven by the activation and recruitment of T cells to the liver. Hence, the ConA model has unique features with respect to its pathogenesis and important similarities to immune mediated hepatitis in humans, such as autoimmune hepatitis, acute viral hepatitis or distinct entities of drug toxicity leading to immune activation. However, the ConA model has considerable variability, depending on the preparation of the compound, genetic background of the mice, sex, age and microbial environment of the animal facility barrier [8].

Summary

Many animal models were developed to study the hepatic diseases. However, no ideal models that cover all the pathological features of these diseases are present. This gap will in turn influence the therapeutic regimen which currently is only diseases minded not patients minded.

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