

REVIEW

An overview of animal models for investigating the pathogenesis and therapeutic strategies in acute hepatic failure

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Abstract

Acute hepatic failure (AHF) is a severe liver injury accompanied by hepatic encephalopathy which causes multiorgan failure with an extremely high mortality rate, even if intensive care is provided. Management of severe AHF continues to be one of the most challenging problems in clinical medicine. Liver transplantation has been shown to be the most effective therapy, but the procedure is limited by shortage of donor organs. Although a number of clinical trials testing different liver assist devices are under way, these systems alone have no significant effect on patient survival and are only regarded as a useful approach to bridge patients with AHF to liver transplantation. As a result, reproducible experimental animal models resembling the clinical conditions are still needed. The three main approaches used to create an animal model for AHF are: surgical procedures, toxic liver injury and infective procedures. Most common models are based on surgical techniques (total/partial hepatectomy, complete/transient devascularization) or the use of hepatotoxic drugs (acetaminophen, galactosamine, thioacetamide, and others), and very few satisfactory viral models are available. We have recently developed a viral model of AHF by means

of the inoculation of rabbits with the virus of rabbit hemorrhagic disease. This model displays biochemical and histological characteristics, and clinical features that resemble those in human AHF. In the present article an overview is given of the most widely used animal models of AHF, and their main advantages and disadvantages are reviewed.

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Key words: Acute hepatic failure; Surgical models; Chemical models; Viral models

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INTRODUCTION

Acute or fulminant hepatic failure (AHF) is a severe liver injury accompanied by hepatic encephalopathy which causes multiorgan failure with an extremely high mortality rate, even if intensive care is provided. Management of severe AHF continues to be one of the most challenging problems in clinical medicine^[1]. Liver transplantation has been shown to be the most effective therapy, but the procedure is limited by shortage of donor organs combined with the disadvantage of needing immunosuppressant treatment^[2,3]. Survival rates are substantially improved today compared with the mortality rate that approximated 100% when the

syndrome was first described nearly five decades ago. Nonetheless, survival has plateaued in recent years, prompting us to consider whether major new advances in disease understanding are needed to further improve the overall outcome^[4].

Since 1970, when Trey and Davidson^[5] introduced the term fulminant hepatic failure, various authors have suggested different classifications aimed to establish prognosis and adequate therapeutic strategies. These classifications are fundamentally based on time elapsed from onset of clinical symptoms or jaundice to the development of encephalopathy^[6-9]. The causes of AHF are varied and in many patients remain unknown. A 6-year study (1992-1998) carried out in Spain into the causes of AHF indicated that the basic etiopathogenic agents of AHF were viral hepatitis (39%), unknown cause (30%), toxins or drugs (21%) and others (10%)^[10]. Acute viral hepatitis constitutes a frequent cause of AHF^[7,11,12]. The viruses causing hepatitis A, B and E are capable of producing AHF, which is rarely seen with hepatitis C virus. The hepatitis B virus is the main causal agent worldwide, responsible for 70% of all cases of viral origin^[13]. The hepatitis E virus causes AHF principally in women in their third term of pregnancy. Other viruses involved comprise the herpes virus, varicela-zoster, cytomegalovirus, Epstein-Barr virus, human herpes type 6, adenovirus and paramyxovirus, mainly in the setting of immunosuppression^[14]. Non-steroidal anti-inflammatory analgesic and anti-bacterial drugs are among the pharmaceuticals which most frequently trigger AHF^[7,15-17]. Other less common causes of AHF include pregnancy, veno-occlusive disease, Budd-Chiari syndrome, Wilson's disease, hemochromatosis, tumoral metastases, sepsis, ischemia and hepatic transplant failure^[18,19].

Etiologies also vary worldwide with considerable differences apparent between Western countries and the developing world. In Europe and North America a large proportion of cases are due to acetaminophen and to idiosyncratic drug reactions^[20], whereas reports from emerging countries in Asia and Africa feature viral illnesses, particularly hepatitis B and E^[17]. The resulting clinical picture is remarkably similar across the different etiologies, reflecting common patterns of response of the innate immune system and the resulting inflammatory response^[21]. Determining etiology is important for two reasons: specific antidotes or therapies may be indicated once the diagnosis is known, and knowing the cause provides a reasonably valid guide to predicting outcome.

The AHF syndrome occurs as a result of the functional failure of a large part of the hepatic parenchyma, and severity is proportional to the level of hepatic damage. AHF provokes profound physiological alterations characterized by encephalopathy, hemodynamic changes and coagulopathy, with frequent development of cerebral edema and renal failure^[14,22,23]. Diagnosis is based on biochemical and hematological data indicating hepatic cell hypofunction. Although prolonged prothrombin time not corrected by vitamin K and impairment of factor V are

widely used, research into prognostic indexes remains an open field of investigation^[24].

The pathophysiology of AHF is an area of great interest. It is evident that a relationship must exist between different pathogenic factors, such as bacteria toxins, cytokines, free radicals and other components of the inflammatory system which cause local lesions^[25]. It seems that the endothelium is the first to release vasoactive agents which affect local and distal blood flow in the critical phase of the disease, with nitric oxide, prostacyclins and endothelins being essential components of the response^[26]. Hyperbilirubinemia is generally conjugated and jaundice is an early indicator which progresses rapidly. Severe coagulation problems arise as a result of a variety of mechanisms. Consumption of factor V indicates hepatic damage regardless of vitamin K levels. Renal failure occurs in 30%-75% of cases, and is associated with a poor prognosis. Thrombocytopenia is also common^[27]. An increase in the plasma concentration of aromatic amino acids (AAA) and normal or slightly elevated values for branched amino acids (ACR) are typical findings in patients with AHF. In fact, a fundamental clinical parameter for AHF is the Fischer index, that is, the ACR/AAA molar ratio, which decreases as the severity of hepatic symptoms develops^[28]. An increase in the amino acids phenylalanine and tyrosine, and a decrease in the Fischer index have been reported in both surgical models^[29] and models using galactosamine^[30].

Intracranial hypertension is a major cause of morbidity and mortality of patients suffering from fulminant hepatic failure. The etiology of this intracranial hypertension is not fully determined, and is probably multifactorial, combining a cytotoxic brain edema due to the astrocytic accumulation of glutamine, and an increase in cerebral blood volume and cerebral blood flow; in part due to inflammation, to glutamine and to toxic products of the diseased liver^[31]. Cerebral edema is a potential life-threatening complication in patients with AHF who progress to grade III/IV encephalopathy^[26]. The current view on the pathogenesis of cerebral edema is that hyperammonemia plays a main role. High arterial ammonia concentrations have been proposed as a predictor of brain herniation and mortality in patients with AFL^[32-34]. Moreover, arterial ammonia concentration, ammonia delivery to the brain, and its metabolic rate are higher in patients with high intracranial pressure, and increased arterial ammonia correlates with increased cerebral flow^[35]. Recent work has also suggested free radical formation occurring at a mitochondrial level as being the potential mediator of cellular dysfunction as opposed to ammonia *per se*^[36].

Research into the molecular mechanisms of hepatic regeneration has aroused wide-spread interest^[37,38]. Although little is still known about the hepatic regenerative process, it is clear that cellular loss and damage in the liver are accompanied by a lack of regenerative activity^[39]. Plasma levels of hepatocyte growth factor (HGF) and transforming growth factor (TGF)- β rise^[40]. An increase in the activity of the

fibrinolytic system, responsible for the activation of both HGF and TGF- β , is also observed^[41]. It has been reported that the serum of people affected by AHF has a negative effect upon culture cell growth when compared with a control serum, due to cell proliferation inhibition rather than to an increase of apoptosis^[42]. Recent studies using various animal models have shown that the over-expression of calpastatin, an endogenous inhibitor of calpain, helps prevent further liver damage when hepatic regeneration is compromised^[43].

Knowledge concerning the pathophysiological basis of the AHF hemodynamic alterations, immunological dysfunction, and multiorgan failure is still very rudimentary. It is therefore crucial to investigate the molecular basis of AHF in more depth^[44]. Furthermore, although many AHF treatment options have been proposed and applied in recent years, only hepatic transplantation is widely accepted among clinical specialists. However, the lack of donors combined with the high costs, technical difficulties, viability issues and the disadvantage of needing life-long pharmacological immunosuppressant treatment following surgical intervention (with the added complication that the immunosuppressant agents used themselves produce side effects in the kidneys, liver and other organs), mean that liver transplantation is not always an option. For these reasons, other therapeutic options to bridge patients to recovery or stabilization have to be considered. Artificial liver support, intended to remove protein-bound toxins and water-soluble toxins without providing synthetic function, and bioartificial liver support systems, using hepatocytes in an extracorporeal device connected to the patient's circulation, are being tested, and molecular adsorbent recirculating systems (MARS)^[45] or cell-based therapies are increasingly the focus of attention^[46,47]. These systems improve clinical and biochemical parameters and can be applied safely to patients^[48], but their effectiveness and viability have not yet been conclusively demonstrated^[58]. In terms of clinical applications, functional studies using animal models are absolutely crucial.

ANIMAL MODELS OF AHF

Knowledge of the pathophysiology and treatment of AHF are limited by the lack of satisfactory animal models. Many attempts have been made to develop a suitable model which can be replicated, using a wide variety of species and approaches, from surgical models to the use of hepatotoxic drugs (Table 1). However, to date a simple model which accurately reproduces the pattern of human AHF has not been reported, and the models currently in use present significant limitations^[49,50].

An ideal model would present well-defined clinical and biochemical criteria, and, as in the case of the King's College AHF prognostic criteria^[11], be capable of providing an accurate prognosis. However, none of the models which have been developed until now meet these requirements. Furthermore, the clinical and biochemical criteria used to indicate the existence of AHF in animal models often have very little in common with those used in clinical practice. However, given the current state of

knowledge concerning AHF and the difficulties involved in carrying out research on patients, animal models have a fundamental role to play in future studies despite their limitations. Therefore, although progress is being made, research in this field must continue, with the aim of developing a reliable and suitable animal model, capable of accurately reflecting the human clinical syndrome and presenting a minimum of disadvantages^[51].

Ideal AHF models, according to criteria widely accepted (Table 2)^[49-54], would benefit from complying with a series of requirements including that the model should be reversible, ie that some animals would survive the process if a suitable treatment were administered, and that the results obtained can be replicated, i.e. that death occurs at recognised intervals and that the extent of hepatic damage can be measured and standardised. Furthermore, death would need to be a result of hepatic damage, i.e. the complications produced following damage would need to accurately reflect the typical human clinical picture and death should be the direct result of the liver damage produced. Therefore, the untreated animals should die with signs of progressive hepatic failure within a recognised period of time. In addition, the animal used would need to be of a size permitting sufficient samples of blood and tissue to be taken during treatment. Finally, all the methods used should represent the lowest possible health risk for personnel participating in the research. An additional criterion could be the use of a conscious animal model to evaluate the development of hepatic encephalopathy, since this is an essential part of the pathology of AHF^[55].

Numerous studies have been carried out in an attempt to develop a suitable AHF model. The majority of animal models are based on surgical techniques or hepatotoxic drugs. Surgical models include the use of hepatic ischemia and partial/total hepatectomy, whilst chemical models are based on the use of drugs and toxins such as acetaminophen, azoxymethane, concanavalin A, galactosamine, halothane, thioacetamide, amatoxin-endotoxin, *etc.* Nevertheless, to date, no model accurately reflects human AHF, and most demonstrate significant limitations.

Surgical models

Surgical models of AHF can be divided into three categories: hepatectomy (total or partial), devascularization (total or partial) and models which are a combination of the previous two.

Total and partial hepatectomy: Surgical models employing total or partial hepatectomy have been successfully developed in various animal species following the first attempt carried out by Mann on dogs in 1921^[56]. It has been demonstrated that 95% liver resection in rats provides a good AHF model^[57], whilst a less than 90% hepatectomy is the upper limit for a liver regeneration research model in mice, as higher values produce mortal hepatic failure^[58]. A potentially reversible model using pigs has been described which combines partial hepatectomy (70%) with porta-caval derivation and produces death from AHF after an interval which is sufficiently prolonged

Table 1 Main AHF animal models in different species

Animal model	Species	Advantages/disadvantages
Surgical		
Total/partial hepatectomy	Pig, dog, rabbit, rat, mouse	Hepatic encephalopathy; reproducible/no reversibility; no long-term survival
Total/partial devascularization	Pig, dog, rabbit, rat	Hepatic encephalopathy; reproducible/no reversibility; no long-term survival
Chemical		
Acetaminophen	Pig, dog, rabbit, rat, mouse	Hepatic encephalopathy; no hazard/non-reproducible; variable interval between damage and death; species and age variability
Amanitin	Pig	Hepatic encephalopathy; specific toxic effects; large animal
Azoxymethane	Mouse	Hepatic encephalopathy; reproducible/small size; hazard
Carbon tetrachloride	Pig, rabbit, rat, mouse	Hepatic encephalopathy/non reproducible; extrahepatic toxicity; small time window before death
Concanavalin A	Rat, mouse	Hepatic encephalopathy/small size
Galactosamine	Pig, dog, rabbit, rat, mouse	Hepatic encephalopathy; biochemical markers/non-reproducible; hazard; variable interval between damage and death; species differences
Lipopolysaccharide	Rat, mouse	Hepatic encephalopathy/non-reproducible; small size; hazard; small time window before death
Thioacetamide	Rabbit, rat, mouse	Hepatic encephalopathy; reproducible; large time window before death/hazard
Viral		
Rabbit hemorrhagic disease	Rabbit	Hepatic encephalopathy; reproducible; no hazard

Table 2 Main criteria for an AHF animal model (according to Terblanche and Hickman (1991))

Reversibility	Suitable treatment may reverse and improve survival
Reproducibility	Reproducible end-points are required to standardize the model
Death from liver failure	Should reflect biochemical, histological and clinical changes including death from AHF
Therapeutic window	Time for treatment should be available between insult and death
Adequate animal size	Size large enough to allow blood and tissue analysis to take place serially
Minimal hazard to personnel	Minimum risk for operators and associated staff

to enable studies of hepatic support measures to be carried out. In addition, the animal is of an appropriate size, and the technique does not represent a health hazard^[59].

The partial hepatectomy models are equivalent to patients who have undergone large liver resections for liver tumors. It has been demonstrated by DNA analyses of rats subjected to various levels of partial hepatectomy that induced AHF is a consequence of both an increased rate of apoptosis and a reduction in liver regeneration^[60]. Moreover, models of partial hepatectomy have been used to test the usefulness of different support systems. Thus, intraperitoneal transplant of syngeneic-bioencapsulated bone marrow cells, which can transdifferentiate into hepatocyte-like cells in the peritoneal cavity of 90% hepatectomized rats, increases the survival rate of these animals^[61]. Examination of the effects of a series of allogenic hepatocyte transplantations in rats with subtotal hepatectomy indicates that intrasplenic hepatocyte transplantation 1 d before liver surgery shows the best results in terms of survival^[62]. The usefulness of an artificial liver module having a liver lobule-like structure has been recently tested in rats with combined partial hepatectomy and hepatic ischemia, demonstrating that in treated

rats the increase in blood ammonia was completely suppressed and all animals recovered^[63].

The clinical equivalent of liver total hepatectomy is the massive liver damage due to liver trauma or a primary graft failure^[51]. Main disadvantages are the absence in circulation of the toxic substances and inflammatory factors which play a role in the pathogenic mechanisms of AHF. Advantages are related to replicability and its usefulness in the *in vivo* study of artificial support devices in the absence of toxic products eliminated or produced by the damaged liver. Despite the disadvantages indicated, total hepatectomy has been used on rats to study hepatic regeneration^[64], and with pigs as a replicable model for testing the effectiveness and function of various temporary support device systems^[65,66]. A new surgical model for hepatectomy in pigs, requiring prior to *en bloc* hepatectomy a Y-shaped bypass starting with end-to-side anastomosis between the vena cava and the portal vein, followed by anastomosis to the intrathoracic vena cava has been recently described. This model permits total hepatectomy with minimal blood loss under stable circulation without requiring an extracorporeal bypass^[67].

Devascularization: Complete devascularization of the liver may be achieved by portacaval shunt followed by occlusion of the hepatic artery, and in most cases also occlusion of the common bile duct and accessory hepatic vessels^[68,69]. Depending on the time of temporary occlusion of the hepatic artery the model is more or less reversible. These techniques have been successfully used to induce a reproducible hepatic failure in pigs, which could be useful in the study of different artificial and/or bioartificial hepatic support devices^[70,71] or to test the effects of antioxidant molecules such as N-acetylcysteine^[72]. For example, a reproducible model has been developed using dwarf pigs for the study of reversible devascularization through hepatic artery ligation and porto-caval anastomosis, where intracranial pressure was monitored in addition to other classic parameters

indicative of AHF. This model provides an 8-h therapeutic window, enabling tests on different bioartificial support systems to be carried out^[73]. In fact, with the use of a similar model in pigs, albumin dialysis using the molecular adsorbents recirculating system (MARS) has been reported to attenuate extracellular brain ammonia and lactate levels^[74]. Hepatic devascularization in pigs has also allowed the demonstration that endothelium-dependent hyperpolarization of vascular smooth muscle contributes to the development of hyperdynamic circulation in AHF^[75].

A model using total clamping of the portal triad in dogs demonstrated that the damage caused by ischemia-reperfusion as a consequence of the surgical procedure was reduced following administration of a bradykinin β_2 receptor antagonist^[76]. Dogs were also used in another AHF model employing porto-caval derivation combined with bile duct ligation, in order to test a new system of bioartificial liver by inoculation of hepatocytes. This model was configured by inoculating porcine hepatocyte spheroids into the cell circuit of a hollow fiber bioreactor^[77]. Recently a new pig model has been developed in which a 75%-80% liver resection is combined with an ischemia period^[78].

Studies carried out on survival time, technical ease, safety and reproducibility of AHF surgical models have reported that devascularization was more useful for studying the development and treatment of AHF caused by ischemia and related side effects, whilst partial hepatectomy was the most suitable technique for studying liver deficiency status and AHF treatment *via* bioartificial support devices^[79].

Chemical models

The use of chemical agents such as acetaminophen, thioacetamide or galactosamine may reproduce a number of important AHF clinical characteristics, such as hypoglycemia, encephalopathy, and increased blood levels of hepatic enzymes, and hepatotoxic chemical agents are still frequently used as a model for AHF. However, repeated administration or a support therapy may be required in some models. In addition, intracranial hypertension, one of the main characteristics of human AHF, is absent in some chemical models whilst in other cases, an increase in toxins involved in hepatic encephalopathy and cerebral edema in human AHF cannot always be demonstrated^[49].

Acetaminophen: Acetaminophen (paracetamol) is a commonly used drug which can produce hepatic damage. In fact, it is the drug most frequently used to commit suicide in the United Kingdom despite the existence of the antidote acetylcysteine. Acetaminophen overdoses are the number one causes of AHF in USA, United Kingdom, and most of Europe, accounting for nearly 50% of USA cases^[13]. Acetaminophen toxicity is dose-dependent, but its effects can be exacerbated by fasting, cytochrome P-450 inducer drugs and especially by alcohol. Studies on both hepatocyte cultures and mice have shown that c-jun kinases (JNK) play a major role in

the toxic effect of the drug^[80]. More recently, it has been shown that apoptosis signal-regulating kinase 1 (ASK1), a member of the mitogen-activated protein kinase kinase family, is activated by acetaminophen overdose in mice, most likely *via* a mechanism involving thioredoxin-ASK1 dissociation, and that it plays a role in acetaminophen-induced liver injury through JNK activation^[81]. The fact that JNK inhibition is not protective in acute carbon tetrachloride-mediated or anti-Fas antibody-mediated hepatic injury, suggests specificity for the role of JNK in the pathogenesis of acetaminophen-induced liver failure, thereby identifying JNK as an important therapeutic target in the treatment of acetaminophen hepatotoxicity^[82].

The results of numerous studies with animal models using acetaminophen to induce AHF have produced heterogeneous results due to the existence of significant variations in the hepatic detoxifying metabolism of the drug related to species and age^[83,84]. Under normal conditions, acetaminophen hepatic metabolism is produced by glucuronidation and sulfation reactions, with formation of metabolites which are later excreted through the kidney. When an excess of the drug is present, normal detoxifying pathways are saturated and the drug is metabolized through cytochrome P-450 to N-acetyl-p-benzoquinoneimine which, unless conjugated with glutathione, is thought to interrupt mitochondrial calcium flux and to induce cell damage by the formation of hydroxyl radicals, nitrites, and nitrates, leading to apoptosis and cell necrosis^[85]. Therefore, in order to potentiate acetaminophen toxicity, inducers of the cytochrome P-450 systems such as phenobarbitone and 3-methylcholanthrene, glutathione depletion induced by the glutathione synthetase inhibitor buthionine sulfoximine or a combination of both systems are used^[84,86].

Other important aspects which have not been standardized in acetaminophen models, and which produce variable results, include the optimal drug dose, the most suitable method of administration and the necessity or not of induction of the cytochrome P-450 system^[86,87]. Lack of standardization is the origin of some of the major disadvantages of these models, specifically, their lack of reproducibility and the variable interval between inducing damage and the death of the research animals^[49,52]. Furthermore, in some rodents significant differences have been found in concentrations of the main coagulation factors compared to those found in human AHF^[88].

Acetaminophen-induced animal models of AHF are widely used to improve our insight into the metabolic and physiological derangements of AHF and to facilitate the development of new therapeutic modalities. Thus, implantation of encapsulated lentivirally immortalized human hepatocytes rescue mice from lethal doses of acetaminophen, confirming that lentiviral vectors represent tools of choice for immortalization of non-dividing primary cells and that immortalized human hepatocytes are promising reagents for cell-based therapy of acute liver failure^[89]. More recently, it has

been found that adult-derived mononuclear bone marrow fraction is capable of significantly increasing the survival rate of rats with acetaminophen-induced AHF^[90]. Research has also shown that acetaminophen-induced hepatocellular damage is associated with increased circulating catecholamines, which may contribute to the pathophysiology of acetaminophen-induced hepatotoxicity by compromising hepatic perfusion, and that toxicity may be abolished by the use of $\alpha(1)$ antagonists^[91].

Galactosamine: D-galactosamine is a molecule which, when metabolized *via* the galactose pathway in the liver, causes serious metabolic alterations and hepatic necrosis through depletion of different uridine intracellular mediators^[49], and has therefore been used to develop AHF models. In one of the first models using rabbits^[92], death occurred between 21 and 44 h, following a coma lasting on average 2.6 h, with histologic and biochemical findings compatible with AHF. Furthermore, it was possible to show that in this same species, hepatotoxin did not cross the hematoencephalic barrier^[93]. More recently, galactosamine has been used on anesthetized dogs. This model also displays the characteristic effects of human AHF, such as an increase in blood levels of liver enzymes, bilirubin, ammonium or lactate and the associated coagulopathy, hypoglycemia, coma and increase in intracranial pressure^[94]. However, the effects were not the same in dogs without anesthesia, probably due to the added effect of the anesthetic. A reproducible model has been developed with pigs which, because of their size, are suitable for the assessment of different support systems designed for treating AHF in humans^[95]. Significant differences in galactosamine sensitivity across different species exist. Furthermore, the interval between damage caused and death is not uniform, the agent is expensive to use in large-scale models, and lastly, it carries health risks^[50].

Galactosamine models have been used to investigate the renal damage which accompanies AHF^[96] and the liver metabolic pathways involved^[97]. In addition, the potential protective effects of substances such as the chimeric protein hyper-IL-6^[98] or 1,6 diphosphate fructose have been investigated in rats^[99]. Cardiostrophin 1 may improve the outcome of D-galactosamine-induced AHF through its effects on anti-apoptosis and cell repair^[100]. Blocking of N-methyl-D-aspartate receptors prevents ammonia-induced death^[101] and also prevents or delays death of rats by galactosamine-induced AHF^[102]. Moreover, this model has been used to identify the contribution of cytosolic polypyrimidine tract-binding protein to the mechanisms of hyperinsulinemia by stabilization of mRNA encoding insulin and its secretory granule proteins^[103]. D-galactosamine models have also allowed testing of different extracorporeal hepatic support devices^[104] and bioartificial systems, including hepatocytes transfected with the human gene interleukin-1 receptor antagonist in rats^[105], the use of a nonwoven fabric bioreactor containing porcine hepatocytes^[47], or the study of the

potential effects of cerebrospinal fluid drainage and cranial decompression in rats^[106].

A combination of D-galactosamine and lipopolysaccharide has also been widely used to induce AHF in rats. This model has allowed the demonstration of the potential therapeutic role of vascular endothelial growth factor^[107]. Using this approach, evidence for a direct link between tumour necrosis factor (TNF)- α and Fas/FasL in mediating hepatocyte apoptosis has been provided^[108], it has been reported that type I inositol 1, 4, 5-triphosphate receptors increase in the kidney^[109], and it has been demonstrated that transcription factor early growth response (Egr)-1 plays an important role in acceleration of hepatic inflammation, apoptosis, and subsequent mortality in acute liver injury^[110]. Research with this model has also found that the expression and activity of both leukotriene C4 synthase and microsomal glutathione-S-transferase are up-regulated, being partly responsible for cysteinyl leukotriene hepatic accumulation^[111], and that a combination of 5-hydroxyindole acetic acid, glucose, β -hydroxybutyrate, and phosphate concentrations in the plasma is a potential marker for AHF, as well as for the early prognosis of AHF^[112]. Studies using SP600125, a small molecule JNK-specific inhibitor have confirmed the role of JNK as a critical apoptotic mediator in galactosamine/lipopolysaccharide-induced AHF^[113]. Very recently it has been demonstrated that in mice challenged with D-galactosamine and lipopolysaccharide, deficiency of uncoupling protein-2, which plays a role in liver cell death through its involvement in the production of reactive oxygen species and adenosine, provides protection under endotoxemic stress conditions, underlining the significant role of the bioenergetic status in critical illness^[114].

Carbon tetrachloride: Carbon tetrachloride has been widely used to induce chronic liver damage, especially as a model of primary hepatic cirrhosis. Nevertheless, its use to induce AHF has been very limited due to low reproducibility and wide variation between species^[50,115]. The mechanism of action is produced in the endoplasmic reticulum by formation of reactive intermediates through isoenzymes of cytochrome P-450^[116]. This mechanism also involves significant alterations to mitochondrial calcium homeostasis and is dose-dependent^[117].

A relatively uniform model was developed using pigs which induced coma and death between 12 and 52 h through a combination of pretreatment with phenobarbital and a 2-h interruption of arterial blood flow followed by intragastric administration of the toxin^[118]. The administration of carbon tetrachloride in rats has been shown to simultaneously induce both severe damage processes and hepatic regeneration^[119]. Depending on the dose administered, exposure time, the presence of exacerbating agents, or the age of the organism affected, regeneration can occur and lead to the total recovery of the damaged liver^[120,121].

Rats have been used for the study of intrasplenic transplant of hepatocytes^[122], and to investigate the mechanisms involved in compensatory liver regeneration which avoids progressive toxic damage^[43]. Carbon

tetrachloride-induced AHF has also allowed the demonstration in rats of the therapeutic efficacy of Gabexate mesilate, a synthetic protease inhibitor^[123], the sulfated polysaccharide extracted from brown algae fucoidan^[124], or naringenin-loaded nanoparticles^[125], but not of granulocyte colony stimulating-factor^[126].

Criticisms of these models include the fact that carbon tetrachloride mainly affects the central zone of the hepatic acinus, and the characteristic massive necrosis of human AHF is not present. Furthermore, carbon tetrachloride is not completely metabolized in the liver and some of the non-metabolized toxin affects and damages other organs, especially the lungs and kidneys^[52]. Finally, there is a wide variation in species and age sensitivity, basically due to different levels of development and effectiveness of the cytochrome P-450 detoxifying system^[49].

Thioacetamide: Thioacetamide causes hepatocellular necrosis following biotransformation by mono-oxygenases^[127], and has been used to explore the role of reactive oxygen species^[128], and the protective effect of antioxidants such as curcumin^[129], pro-regenerative substances^[130], or the worsening of encephalopathy following long-term treatment with substances such as indometazine^[131]. Using the thioacetamide model of AHF, it has been recently shown that cannabinoids and capsaicin improve liver function^[132] and that *Ginkgo biloba* ameliorates hepatic damage most probably due to its free radical-scavenging effects^[133]. Simvastatin improves encephalopathy and survival in thioacetamide-treated rats, an effect that is offset by N(G)-nitro-L-arginine methyl ester (L-NAME), a non-selective inhibitor of nitric oxide synthase (NOS), which supports the role of nitric oxide in liver damage and encephalopathy^[134]. Moreover, the fact that L-NAME administration, but not L-canavanine (specific inhibitor of inducible NOS), had detrimental effects on the severity of hepatic damage and motor activities in thioacetamide-treated rats, suggests that constitutive NOS activities play a major protective role^[135].

Azoxymethane: Azoxymethane administration induces in mice alterations similar to those encountered in human AHF^[136]. In fact, it has been shown that mice present decreased locomotor activity followed by loss of righting and corneal reflexes, are hyperammonemic, and develop spontaneous hypothermia and brain amino acid profiles typical of AHF in other species including humans. These findings demonstrate that azoxymethane treatment affords a reproducible model which may be suitable for the study of the cerebral complications of AHF^[137]. Induction of AHF in C57BL/6J mice by using azoxymethane has recently allowed the observation that altered expression of zonula occludens-2 precedes increased blood-brain barrier permeability, suggesting that zonula occludens-2 may play an important role in the pathogenesis of brain edema in AHF^[138].

Concanavalin A: A single injection of concanavalin A has also been proposed as a model of AHF as it induces

hepatocellular destruction^[139] through mechanisms which appear to involve participation of immune cells, including macrophages and activated CD4+ T cells^[140]. The use of this animal model has demonstrated that suppressor of cytokine signaling-1 (SOCS1) plays an important negative role in fulminant hepatitis and that forced expression of SOCS1 is therapeutic in preventing the disease^[141]. In concanavalin A-treated mice it has been reported that TNF- α levels are not affected by adiponectin, whereas IL-10 production is increased. Therefore, adiponectin might play a role in the control and limitation of inflammation in the liver, and a contribution has been suggested for IL-10 in adiponectin-mediated hepatoprotection^[142]. siRNA delivery for osteopontin, which has been implicated in various helper T cell type 1 immunity-mediated diseases, has therapeutic potential in concanavalin A-mediated AHF^[143].

Other models: AHF has also been induced through the use of poisons such as the derivative of *Amanita phalloides* which, although not a frequent cause of poisoning, has well-known effects on humans. In fact, the effect of amatoxins is due to ARN polymerase induction, producing cell toxicity in hepatocytes, intestinal mucosal cells and kidney tubular cells and they have been used in combination with lipopolysaccharide to develop an animal model of AHF using pigs^[144]. Models with pigs have also been reported which combine amanitine with lipopolysaccharide, with the aim of studying survival following orthotopic liver transplant and tacrolimus administration^[145].

Other models employ parenteral administration of *Propionibacterium acnes* and lipopolysaccharide in mice to study the inhibition of the acquired immune response^[146], and intraportal administration of α -amanitine and lipopolysaccharide in pigs to study bioartificial liver support devices^[147].

Intrahepatic upregulation of the immunostimulating molecules CD40 and CD40 ligand (CD40L) are early mechanisms for liver cell damage in human and murine AHF. The use of a model based on intrahepatic overexpression of CD40L by adenoviral-mediated gene transfer (AdCD40L) in mice, has led to the demonstration that CD40-CD40L interaction can induce liver damage, and that CD40L-induced AHF depends on competent lymphocytes^[148].

Although their use has been limited, various models combining drug administration and surgical procedures have been described, such as a combination of 70% liver resection and endotoxin administration in rats^[149], or resection of all of three hepatic arteries combined with intraportal injection of carbon tetrachloride^[150].

Viral models

Despite the fact that viral hepatitis is a main cause of AHF in many countries, the use of infective agents to develop animal models of AHF has in general been unsuccessful and only the use of transgenic mice overexpressing virus B hepatitis proteins (HBV) or BALB/cj mice infected with MHV-3^[151,152] has shed some light

on virus-induced AHF mechanisms. However, these murine models display significant limitations as regards the absence of intracranial pressure measurements, or the lack of data concerning toxins involved in hepatic encephalopathy and cerebral edema, as well as the small size of the models used which renders testing of new liver support systems impossible^[49,50].

More recently, our research group has described a new animal model of AHF using experimental infection of rabbits with 104 hemagglutination units of an isolate of the rabbit hemorrhagic disease virus (RHDV)^[153]. First reported two decades ago, RHDV is a member of the *Caliciviridae* family which causes an acute and highly fatal disease in wild and domestic rabbits^[154]. Rabbit hemorrhagic disease (RHD) is a viral hepatitis which displays surprising clinical, anatomopathological and transmission mode similarities to fulminant human viral hepatitis B, C, and E^[155]. The virus does not replicate in any other vertebrate^[156] and to date there is no indication that it can be transmitted to humans, even among those populations most exposed to the virus.

It has been shown that the viral antigen can already be found in hepatocytes at 12 h postinfection (p.i.) and that at 36 h and 48 h p.i., it is localised in 60%-80% of hepatocytes^[157]. RHD is characterized by a high morbidity and a mortality rate that approaches 90%^[153,158]. Rabbits die within 36 to 54 h p.i. with clinical signs characteristic of progressive AHF and coma. In addition, the interval between infection and death, in the majority of animals, provides a wide therapeutic window which indicates that our model complies with another of the essential prerequisites of a good AHF animal model, that is, the existence of a sufficiently prolonged interval between intervention and death to enable research into various treatment methods or liver support technologies. In addition, the use of a medium-sized animal facilitates serial collection of blood samples, and makes easier monitoring of intracranial pressure and biochemical alterations produced during the course of the infection^[153].

This model reproduces representative biochemical and histological parameters and clinical signs of human AHF. Thus, significant increases in blood transaminase and lactate dehydrogenase activities, and in blood bilirubin concentrations, are detected. Moreover, blood concentration of aromatic amino acids increases significantly, with a decrease in the Fischer index and hypoglycemia. Prolonged prothrombin time, a prognostic element in AHF, and exhaustion of factor V and VII are systematic findings. These effects could occur as a consequence of diminished synthesis of clotting factors and the development of disseminated intravascular coagulation^[153]. In addition to biochemical and histological abnormalities, infected rabbits demonstrate a clinical picture consistent with AHF. Prostration and side recumbency are present at later stages and neurologic symptoms (convulsions, ataxia, and posterior paralysis) rapidly progress to coma and brain death in the terminal phases. In our model, intracranial pressure rises progressively

in the terminal phases, suggesting a loss of intracranial compliance, and short episodic spikes are also observed. The rise in intracranial pressure in RHDV-infected animals is accompanied by an increase in plasma ammonia levels^[153].

Histological and immunohistochemical examination reveals necrotic areas associated with hemorrhages and neutrophil infiltration, and large apoptotic areas with a high caspase 3 expression, mainly in the periportal areas of hepatic acini^[159]. A significant increase in inducible nitric oxide synthase expression and TNF- α activity, similar to those reported in AHF^[160], are also observed in infected rabbits^[161]. TNF- α may lead to cell proliferation or to apoptosis, and its over-expression correlates with both apoptosis and hepatic regeneration in AHF^[162]. Balance between proliferation and apoptosis may be influenced by an excess of reactive oxygen species that, if not neutralized by glutathione and antioxidant enzymes, may cause mitochondrial damage and cytosol release of cytochrome c, causing caspase activation and cell death^[163]. This also happens in RHDV-infected rabbits, which show impaired glutathione levels and antioxidant enzyme activities^[161], with a marked activation of the apoptotic intrinsic pathway^[159].

Therefore, RHDV experimental infection induces an AHF in rabbits which has a number of physiological and biochemical features seen clinically in humans, is highly reproducible, has a long therapeutic window and generates intracranial hypertension and an associated-encephalopathy. Thus, it is the first successful model using infective agents and satisfies the criteria applicable to an animal model of AHF. This model could provide a useful tool for the study of AHF and the evaluation of new liver support technologies in humans.

CONCLUSION

AHF is a potentially devastating syndrome whose treatment has been limited by the lack of satisfactory animal models. The potential disadvantages of surgical models are that they do not offer reversibility or recovery, they are difficult to replicate, they depend on surgical skill, many of the clinical and biochemical parameters typical of human AHF are not present, and that they do not reproduce an environment complicated by the release of inflammatory mediators and products of cell necrosis. Thus, their usefulness is limited to the evaluation of various liver support systems. Models using hepatotoxins do not suffer from the above limitations, but nevertheless they may present disadvantages, such as the necessity for adjusting dosage and the potential health hazard which in most cases such chemical agents represent. As for the only viral model developed to date which has proved to be viable, induced RHDV infection in rabbits, it is reproducible and presents characteristics similar to human AHF. The only limitation is that the only susceptible species is the rabbit, although this could also be considered an advantage as it does not represent a health hazard to researchers.

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