Research progress on hepatic encephalopathy: animal models and disease mechanisms

Jingjing Li¹, Jia Xiao²,³, Kwok-Fai So¹

¹GMH Institute of Central Nervous System Regeneration, Jinan University, Guangzhou, China
²State Key Discipline of Infectious Diseases, Department of Infectious Diseases, Shenzhen Third People’s Hospital, Shenzhen, China
³Department of Immunobiology, Jinan University, Guangzhou, China

Correspondence: Jia Xiao or Kwok-Fai So
E-mail: tjxiao@jnu.edu.cn or hrmaskf@hku.hk
Received: April 01, 2015
Published online: April 13, 2015

Hepatic encephalopathy (HE) is a hepatic disease with neuronal confusion, altered level of consciousness, and coma as a result of severe liver damage/failure. HE is with complicated pathological mechanisms and high mortality, which seriously affects patients’ daily life. Roughly, it can be divided into three types, A, B and C. Type A is acute HE and Types B and C are chronic. At present, the pathogenesis of HE is still unclear. In addition, there is no specific clinical treatment. Therefore, establishing appropriate HE animal model is vital for the mechanistic study of the disease and the development of clinical therapy. This review makes a summary on the recent progress of HE animal model establishment and current study of its underlying molecular mechanisms.

Keywords: hepatic encephalopathy; animal model; disease mechanism

To cite this article: Jingjing Li, et al. Research progress on hepatic encephalopathy: animal models and disease mechanisms. Abdomen 2015; 2: e770. doi: 10.14800/Abdomen.770.

Introduction

Hepatic encephalopathy (HE), also known as portosystemic encephalopathy, is a hepatic dysfunction syndrome with metabolic disorders of the central nervous system (CNS) and caused by severe liver diseases or portosystemic shunt [1]. Clinical HE is divided into three categories: Type A: HE associated with acute liver failure, not including acute HE with concomitant chronic liver disease; Type B: HE related portosystemic shunt, but no parenchymal injury of the liver; Type C: HE associated with cirrhosis, portal hypertension and (or) portosystemic shunt [2]. Common neurological manifestations of HE mainly include blurred consciousness or coma, cognitive dysfunction, hyperreflexia, slow monotonous speech, loss of motor skills, asterixis, clonus, hyperventilation and seizures [3]. To date, the molecular pathogenic steps of HE development remain unclear. Besides that, no clinically effective treatment has been confirmed for this disease. The individual condition of patients is quite different and this will seriously affect their prognosis. Thus, HE has been a research focus in the fields of hepatology and neurobiology for a long time. Establishing appropriate animal models of HE with 3R requirements (replacement, reduction and refinement) is the basis to study its mechanisms. They can therefore provide an effective route for the development of novel but effective clinical diagnosis and treatment. This article reviews the research progress of disease progressive mechanisms and selection guide of experimental animal in establishing animal models of different types of HE.

Selection of experimental animals for HE study

At present, many species of animals have been used to
Failure or type A HE is limited. Liver failure patients in clinic. Given these limitations, the physiological changes between this animal model and acute also have some differences of pathological and models, these substances are rarely enter blood circulation, which has a certain impact on other organs of the patient. Due to hepatic blood flow is completely blocked in anhepatic models, these substances are rarely enter blood circulation, so there are some differences of pathological and physiological changes between this animal model and acute liver failure patients in clinic. Given these limitations, the application of anhepatic models in studying acute hepatic failure or type A HE is limited.

In addition to anhepatic models, partial hepatectomy is also a method to induce type A HE. Panis et al. hypothesized that liver failure caused by partial hepatectomy may be due to progressive damage in the remnant liver. Since a positive correlation between the speed of liver regeneration and the percentage of liver resection (the greater proportion of resection to be performed, the faster that liver regenerate), the consistency of injury degree in partial hepatectomy is difficult to control, and the surgery often failed because of intraoperative blood loss. In addition, drinking and food consumption of animals cannot be restored immediately after the surgery, resulting in severe hypoglycemia, making the animal died even more quickly. Due to those disadvantages, partial hepatectomy is only used by few researchers under certain circumstances.

Additionally, hepatic vascular occlusion is used to establish the model of type A HE. This method is divided into two categories: hepatic inflow and inflow/outflow, through portacaval shunts and hepatic artery ligation. This approach makes the liver cells tend to be necrotic, so as to achieve the purpose of constructing the model of liver injury. After the portacaval shunt, the hepatic blood flow can be entirely or partially according to the actual operating situation. After full hepatic vascular occlusion, the hepatic coma of animal model can be consistent with clinical situation. After partial hepatic vascular occlusion retains the damaged liver, and the reversibility of liver dysfunction can be controlled by blocking time. It overcomes the shortcomings of full hepatic vascular occlusion, which is suitable for long time experiment and observation. So in practical application, partial hepatic vascular occlusion is a better surgical operation to induce the animal model of type A HE.

Hepatotoxic drugs

1. Thioacetamide (TAA)

TAA has been shown to be an effective drug of inducing an animal to produce different grades of liver injury (including nodular cirrhosis) through the inhibition of respiratory metabolism in the liver. The specific mechanism is the aggregation of calcium ions within the cell membrane, which affects the permeability of the membrane, and then to inhibit the oxidative phosphorylation in the mitochondria and cause liver metabolism disorder. Some studies have shown that partial liver cell injury induced by TAA is completed with oxidative stress, which was mediated by cytochrome P450-induced lipid peroxidation. In addition, after TAA treatment, the content of ammonia in serum and brain tissue is also increased.

Table 1. Animal models of type A hepatic encephalopathy (HE)

<table>
<thead>
<tr>
<th>Induction method of HE</th>
<th>Species used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anhepatic models</td>
<td>Rat, rabbit, pig</td>
</tr>
<tr>
<td>Hepatectomy</td>
<td>Rat, pig</td>
</tr>
<tr>
<td>Portacaval anastomosis + ammonia</td>
<td>Rat</td>
</tr>
<tr>
<td>Hepatotoxin models</td>
<td>Rat, rabbit, guinea</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>Rat, rabbit, guinea</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Rat, dog, pig</td>
</tr>
<tr>
<td>Thioacetamide</td>
<td>Rat</td>
</tr>
<tr>
<td>Azoxymethane</td>
<td>Mouse</td>
</tr>
</tbody>
</table>

Table 2. Animal models of type B or C hepatic encephalopathy (HE)

<table>
<thead>
<tr>
<th>Induction method of HE</th>
<th>Species used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portacaval anastomosis</td>
<td>Rat, dog, rabbit, pig</td>
</tr>
<tr>
<td>Congenital portacaval shunts</td>
<td>Dogs, cats</td>
</tr>
<tr>
<td>Graded portal vein stenosis</td>
<td>Rats</td>
</tr>
<tr>
<td>Portal vein stenosis + ammonia</td>
<td>Rats</td>
</tr>
<tr>
<td>Bile duct ligation*</td>
<td>Rats</td>
</tr>
</tbody>
</table>

*Manifests several aspects of type C HE

Due to large animals are more expensive and difficult to manage and feed, as well as the ethical problems, majority of current studies tend to use small animals (primarily mice and rats) which are cost-effective and practical with wide availability of sources.

Type A HE: animal models and pathogenic mechanisms

Anhepatic models

Animal models of type A HE can be established by inducing acute liver failure through the anhepatic models (including full or partial hepatectomy liver and hepatic vascular occlusion models). Among those strategies, total hepatectomy undoubtedly can lead to liver failure. However, due to the lack of the entire liver, this model is of poor reversibility, and the biochemical abnormalities only occur shortly before the death of animal (2-4 h) with even shorter coma duration. In addition, when clinical acute liver failure occurs, injured liver tissue still produces a number of toxic substances into the body through the blood circulation, which has a certain impact on other organs of the patient. Due to hepatic blood flow is completely blocked in anhepatic models, these substances are rarely enter blood circulation, so there are some differences of pathological and physiological changes between this animal model and acute liver failure patients in clinic.
of mice significantly increased, which were closely related with its pathological symptoms [including weight loss, blood clotting abnormalities, electroencephalography (EEG) changes and cerebral edema]. It suggests the rise of ammonia content in serum and brain may be an important cause of acute HE [15]. There are diverse administrations for TAA-induced HE, but no consistent dosage has been settled down. In the rat model, common modes of administration include [9]: intraperitoneal injection for three consecutive days of 300 - 350 mg/kg, subcutaneous injection of 200 mg/kg, and intragastric administration two days of 300 mg/kg, etc. These administrations can cause severe liver injury in rats to induced type A HE. A series of clinical trials found that 300 mg/kg/d TAA could successfully establish a HE model [16]. But this animal model will lead to hypoglycemia and electrolyte imbalance in rats. To avoid such situations, the researchers gave the model 10% glucose water mixed with lactate ringer (25 mL/kg) by subcutaneous injection every 12 h after the initial injection of TAA [17]. TAA induced HE by intraperitoneal injection has good repeatability, easy operation, high success rate, and high similarity to the HE phenotypes in human. It is commonly used to induce model nowadays [11]. Our laboratory also found that, intraperitoneal injection once of 400 mg/kg TAA in C57/BL6 mice could cause typical symptom of HE. More than 60% mice died within 2-3 days after injection. The survival mice could alleviate most liver injury and a few injuries of the brain by oneself after a week (Xiao et al., Submitted manuscript).

2. Acetaminophen (APAP)

APAP, also known as paracetamol, is a commonly used clinical antipyretic and analgesic. Its normal doses produce a therapeutic effect on the body, but an excessive use will cause liver damage. After taking normal dose, a small amount of APAP converts into the intermediates NAPQI, which causes the endonuclease to transfer into the nucleus to impair DNA, and eventually lead to necrosis of liver cells and liver function failure [18]. The dose of APAP can be used to establish an animal model of HE is usually an intraperitoneal injection of 300-600 mg/kg or an oral administration of 3 g/kg with an observation time for 24 h [19]. Type A HE model induced by APAP has the advantages of easy preparation, low price and dose-dependence. However, this animal model is of poor reproducibility, and APAP has the side effect to kidneys and other organs [20]. Additionally, the model has some differences with the real situation of clinical acute liver failure. Thus, APAP is not often used to establish type A HE model.

3. D-galactosamine (D-Gal)

The mechanism of hepatic injury caused by D-Gal is thought to be relevant with the capture of uridine diphosphate (UDP). Loss of UDP will seriously affect the energy metabolism of liver cell and the synthesis of DNA, RNA and protein. Some following studies also found that D-Gal had the relationship with the integrity of liver cell membrane, the exhaustion of glutathione and tumor necrosis factor [21]. Many new researches point out that LPS and D-Gal in combination can cause very serious liver injury [22, 23]. In addition, Liu et al. injected D-Gal of 1.2 g/kg, 1.4 g/kg and 1.6 g/kg by intraperitoneally into three groups of female SD rats respectively, and then determined the function of liver by collecting the blood sample every 12 hours after injection [24]. The authors confirmed intraperitoneal injection of 1.4 g/kg D-Gal could simulate the physiological and pathological changes of acute liver failure well and construct a stable animal model of acute liver failure in rats. D-Gal model is of better repeatability, unobvious liver toxicity, and manifestations of liver injury is similar to viral hepatic failure clinically with controlling the dose in a certain range, so it is an ideal type A HE animal model [9]. But several potential limitations, such as high cost, short survival time and poor stability still exist, which need to be improved in future practical application [25].

4. Carbon tetrachloride (CCl4)

CCl4 is a widely used chemical that causes liver injury. Its main mechanism is to be activated in liver microsomes by mixed functional enzymes which are dependent on the cytochrome P450 family, generating a large number of free radicals, causing lipid peroxidation within the cell. In addition, CCl4 can bind with protein covalently through the hepatic microsomal lipid to dissociate the cell membrane structure, causes the increase of membrane permeability [26, 27]. Xiong et al. established a type A HE rat model via ligation, two-thirds of the liver resection induced by CCl4. Chang et al. induced acute liver failure and HE in male BALB/c nude mice by intraperitoneal injection CCl4 of 2.5 mL/kg, and the mice had obvious liver injury and brain dysfunction 1-3 days after injection. They also found that treatment with antioxidant N-acetyl-cysteine (NAC) at the same time could significantly improve the injury [29]. However, high concentration of CCl4 has mild irritation to mucosa, and its narcotic effect on the central nervous system and serious injury to the liver and kidneys ask researcher to pay special attention to the possible health damage when uses this chemical [5,30].

Type B HE: animal models and pathogenic mechanisms

Type B HE model is established mainly through portosystemic anastomosis (PCA) or bile duct ligation (BDL).
Glutamine in the brain tissue, changes circadian rhythm, molecular markers (e.g. sodium fluorescein), then leads to cholic acid in rat plasma, changes the permeability of blood brain barrier (BBB), and influences the pharmacokinetics of addition, recent study shows that short-term (10 d) PCA reflection, reduces the utilization of cerebral glucose and alters the function of a variety of neurotransmitters. In addition, recent study shows that short-term (10 d) PCA operation significantly increases the levels of ammonia and glutamine in the brain tissue, changes circadian rhythm, decreases the ability of motion, memory, learning and reflection, reduces the utilization of cerebral glucose and alters the function of a variety of neurotransmitters. In addition, recent study shows that short-term (10 d) PCA operation significantly increases the levels of ammonia and cholic acid in rat plasma, changes the permeability of blood brain barrier (BBB), and influences the pharmacokinetics of molecular markers (e.g. sodium fluorescein), then leads to the occurrence of HE. Besides rat, pig and dog are also used to establish the animal models, which can better simulate the clinical mild HE phenotypes, but tend to cause severer coma because of hypersensitivity to ammonia.

BDL is capable of causing cholestasis and liver cell injury, resulting in chronic hepatic damages. Zhu et al. studied the dynamic changes of liver and kidney functions through the extrahepatic cholestasis induced by BDL in rats. They found that both liver and kidney functions in BDL rat model was significantly worsened. This phenomenon might be attributed to hyperplasia of bile duct epithelial cells after BDL, which is a key factor and center pathological link to a series of lipid peroxidation reactions. Recent animal study found that de novo synthesis of lactate in rat brain is one of the main reasons for the occurrence of cerebral edema six weeks after BDL. Decreased levels of glutamine synthetase and glutaminase in the liver also contributed to cause hyperammonemia. Because BDL animals often develop jaundice, liver failure, portal hypertension, portal vein shunt, sepsis and immune system dysfunction and other diseases after BDL, those interference factors may affect the final HE phenotypes and only mild encephalopathy is developed. Therefore, BDL is often used in association with other methods (such as partial hepatectomy) to establish a utilitarian type B HE model.

Type C HE: animal models and pathogenic mechanisms

In China, the majority of patients for clinical HE are type C. Clinically, type C patients often recurrently develop personality and behavior changes, accompanied by flapping tremor, muscle tone, tendon hyperreflexia, ankle clonus/positive Babinski syndrome and other neurological abnormalities. The mechanism of type C is primarily attributed to the formation of liver cirrhosis in which a relatively obvious portacaval collateral circulation has already been established. Hepatic function insufficiency and short-circuit of circulation are key causes to the patients with chronic liver disease-induced type C HE, then accumulated toxins will circulate into the brain via the altered BBB and causes encephalopathy by abnormal neurotransmissions. At present, most scholars believe deregulated liver functions and impaired CNS functions can influence each other, resulting in secondary damages to the body. Recent study found that increased activity of brain acetylcholinesterase in patients and animals of type C HE would cause the decrease of acetylcholine level. Interestingly, this phenomenon was not found in the animals with type A or B HE. Since acetylcholine is the inhibitor of HE, and such change is independent of the induction of circulating ammonia level, it is considered to be a new molecular mechanism for type C HE progression.

Type C model is often established by inducing chronic liver cirrhosis using CCl₄, ethanol or other liver toxic substances, in association with portacaval anastomosis. Consecutive intragastric administration of a mixture of 1-2 mL/kg 40% CCl₄ and 4-6 mL/kg 50%-60% ethanol is a common establishing method for type C HE. But so far, there is no satisfactory type C HE animal model induced by alcoholic liver disease or viral hepatitis, which are the most common causes in clinical patients.

Concluding remarks

To date, we have a number of methods to establish the HE animal model. But different clinical types of HE need to be stimulated by specific animal models for the study of pathogenesis and the searching of suitable treatment methods. In recent years, with emerging new animal models, study for novel molecular mechanism, diagnose and therapy have got rapid progresses. Ammonia poisoning is considered to be the main mechanism of all three kinds of HE. Circulating ammonia increases when the liver is of dysfunction. Then high level of ammonia may enter the brain tissue through BBB to disturb the energy metabolism and induce HE. Amino acid metabolic imbalance theory and manganese poisoning theory are other main explanations for the initiation and progression of HE.

Clinically, HE is one of the main causes of death in patients with liver disease, so early diagnosis and treatment are vital for better prognosis and improved survival rate of the HE patients. But currently diagnostic method with high sensitivity and specificity is lacking. The most common way to confirm HE is based on clinical features and laboratory examinations with exclusion of other causes of encephalopathy. For example, bases on acute liver failure,
cirrhosis and (or) an extensive medical history of portosystemic shunt, the performance of abnormal neuropsychiatric and the examination of blood ammonia while excluding other neuropsychiatric abnormalities [36]. Because ammonia poisoning theory is the center position in the pathogenesis of HE, blood ammonia measurement is still the “golden standard” biochemical examination [44]. Results of EEG, psychological test, brainstem auditory evoked potential (BAEP), neuroimaging and other measurements should also be taken into consideration.

We can only take a comprehensive treatment based on the pathogenesis of the HE disease so far, including removal of stimulus, nutrition support, drug therapy and artificial liver support therapy [36].

At present, animal models for type A HE is the most mature. There are many kinds of methods, of which drug TAA-induced acute hepatic failure for the establishment of the model is the most commonly used. However, for type C HE, there is still no suitable animal model, which warrants further deep investigation. The establishment of animal model is not only beneficial to the mechanistic study, but can also provide theoretical and clinical basis for the prevention and treatment of HE, such as the application of novel drugs for the removal of ammonia and research progress of artificial liver support system/liver transplantation [42, 47]. In recent years, some studies combined two or more methods to produce a more clinically relevant model of HE, such as blood flow occlusion combines portacaval anastomosis, or partial liver resection combines portacaval anastomosis, which has proven to be a new direction for the future exploration [9].

References


