Hepatic encephalopathy is a neuropsychiatric syndrome associated with acute liver failure, chronic parenchymal liver disease, or portosystemic anastomosis. Many approaches have been used to develop suitable models of hepatic encephalopathy, each with specific advantages and disadvantages. The most commonly used models have been the surgical hepatectomy and liver devascularization procedures, and hepatotoxins such as galactosamine and acetaminophen. Drug-toxicity models may be clinically more relevant. The specific requirements of experiments to study a particular aspect of encephalopathy also influence the choice of animal model. Animal models will play a central role in future research into hepatic encephalopathy to better understand its pathophysiology and to develop newer therapeutic modalities for this condition. [Indian J Gastroenterol 2003;22(Suppl 2):S33-S36]

**Key word:** Acute liver failure, cirrhosis, hepatotoxin, portosystemic shunt

Experimental systems are used to unravel the complex cellular interactions involved in the pathogenesis of a disease. The choice of an experimental system influences the nature of data that can be generated and places certain limitations on the interpretation of those data. In vivo systems that involve the whole animal provide the most natural experimental conditions. However, due to their complexity, these systems have an array of unknown and uncontrollable cellular interactions that may render the interpretation of data difficult. At the other extreme are the *in vitro* systems in which defined populations of cells are studied under controlled and consequently repeatable conditions; these systems can be simplified to the extent that individual cellular interactions can be studied effectively. Yet, *in vitro* systems have their own limitations, the most notable of which is their artificiality.

Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome associated with acute or chronic hepatocellular failure, increased portosystemic shunting of blood, or both. HE complicating acute liver failure is referred to as fulminant hepatic failure (FHF). Pathogenetic mechanisms underlying HE have been extensively investigated using animal models of HE, or cultures of central nervous system cells treated with neuroactive substances that have been implicated in HE.

**Need for animal model of HE**

Over the last decade, several treatment options have been suggested for HE. These include liver transplantation, bioartificial liver support systems, and hepatocyte transplantation. An ideal animal model should induce hepatic failure that is potentially reversible, produce reproducible liver damage, show selective liver damage that leads to death from liver failure during an interval similar to that in clinical situations, die at a time that is long enough from insult to provide a suitable therapeutic window, be a large animal to allow the use of therapies that are applicable to man, use a safe toxin in order to minimize hazard to laboratory staff, be similar in metabolite and physiological properties, so that the response to surgery or toxic insult is similar, and comply with strict ethical and animal-welfare standards. Also, the animal should be genetically well characterized, in order to control experimental variation caused by differences in the genetic background.

Animal models of HE that have been developed till date are either surgical or drug-induced.

**Surgical models**

Elimination of liver function can be carried out either by removal of the liver (anhepatic animals) or by removal of blood supply to the liver (total devascularization). A recent study on dogs and cats reported the use of portosystemic shunts ligated over gauged stainless steel rods.

Anhepatic models: Total hepatectomy involves greater surgical trauma than other surgical modes of acute liver failure, which may alter the cause of subsequent liver failure. Filippo et al described a one-stage minimally traumatic total hepatectomy with minimal variation in hemodynamic and hematologic parameters, with no requirement for transfusion. Such models have used different species, like rats, rabbits, dogs and pigs. Total hepatectomy causes absolute liver failure and produces a short period of coma associated with biochemical abnormalities 2 to 4 hours before death. Olafsson et al showed an increase in intracranial pressure (ICP) in rats undergoing hepatectomy after portocaval anastomosis (PCA) compared with control rats or those that had undergone PCA alone. A major concern about these models is the absence of injured or necrotic liver cells. In comparison, in man, the damaged liver is still per-
fused with blood, allowing the entry of toxic substances into the circulation. Thus, anhepatic models have had a limited role in the study of acute liver failure.

**Devascularized models:** Most models are created in a two-stage procedure - PCA followed by hepatic artery ligation; the delay between the two varies from one model to another. A longer delay is associated with more gradual onset of liver failure and death. To increase the potency of hepatic devascularization, Rozga et al. also ligated the gastroduodenal and accessory arteries, because of concern that retrograde flow through these vessels was associated with recovery of liver function.

A combination of clinical parameters, such as coma and measurement of ammonia, prothrombin time, liver function tests, and glucose, allows for confirmation of liver failure. The recognition that fatal elevations of ICP occurred in FHF led to a significant change in the understanding of this condition. Changes in electroencephalogram (EEG), notably progressive increases in amplitude and frequency, paralleled the deepening of coma and were similar to those seen in humans. There was, however, no temporal relationship between deterioration in the pattern of EEG recordings and ICP levels. Necropsy in rats and pigs confirmed the presence of swollen brains, with evidence of electron microscopic enlargement of astrocytic foot process. Levels of cerebral water and glutamine content were increased in rats undergoing hepatectomy and hepatic devascularization.5

As in the anhepatic models, the complete devascularization produced by permanent hepatic artery ligation is irreversible. Graded devascularization models constitute the most satisfactory surgical models at present. Liver biopsy specimens showed that 25% of liver tissue was not necrotic and thus offered the potential for recovery. Further studies are however required before such a model can be described as reproducible.

Blood pressure and temperature need to be controlled in models. Most models maintain blood pressure by means of fluids and isotropic support. The temperature control in a given model must be within a narrow range. Olafsson et al.3 controlled temperature at 37.5°C ± 5.0°C, whereas Nyberg et al.6 stipulated a more controlled range of 36°C to 37°C. Moderate hypothermia (32°C) may be of benefit in the treatment of uncontrolled intracranial hypertension in patients with FHF.8

To account for the effect of anesthesia and laparotomy in an animal model, it is imperative that appropriate control experiments are undertaken. Except for Rozga et al.,7 most groups have performed control experiments, either sham laparotomies or PCA. Conjeevaram et al.9 have reported reversal of behavioral changes in rats with portacaval stent on neomycin therapy. The antibiotic was given orally. Reversibility has been a demerit with surgical models. Benoist and co-workers10 have made an effort to reverse acute hepatic failure in pigs.

Early surgical models had several drawbacks; refined graded devascularization models appear to more closely mimic the inflammatory changes that occur in liver failure. Surgical models do have a significant role in the testing of artificial liver devices, as chemical models are not so reproducible.

**Chemical models**

Many hepatotoxins, such as carbon tetrachloride, thioacetamide, nitrosamines, halogenated anesthetics, galactosamine, and acetaminophen have been described in the literature.

**Galactosamine models:** D-galactosamine, a selective hepatotoxin, is an amino sugar metabolized by the galactose pathway in the liver. It depletes intracellular uridine moieties, in turn disturbing hepatocyte RNA metabolism, leading to hepatocyte necrosis. Models based on both small and large animals have shown development of FHF in a reproducible fashion after galactosamine administration.11 Extensive injury, specifically necrosis in central and intermediate zones, portal and periportal hemorrhage, and panlobular reduction in hepatocyte glycogen content was seen on histology.

**Acetaminophen model:** In therapeutic doses, acetaminophen is detoxified by a combination of glucuronidation, sulfation, and renal excretion. On overdose, these pathways become saturated and a greater proportion of acetaminophen is metabolized through the P-450 system, leading to production of the toxic metabolite, N-acetyl parabenzoquinoneimine (NAPQI).12 Glutathione (GSH) detoxifies NAPQI and thereby protects hepatic cellular constituents from its direct toxic effect. Walker et al.13 found single cell necrosis at 3 hours followed by the characteristic picture of centrilobular necrosis at 6 hours in liver of male Swiss white mice after acetaminophen administration. Although satisfactory for pathophysiology, such models are less satisfactory for testing the efficacy of bioartificial liver devices. To potentiate acetaminophen toxicity, Miller et al.14 used phenobarbitone, which increased liver damage and decreased animal survival. Cytochrome P-450 levels were found to be higher in animals receiving phenobarbitone. An acetaminophen dose of 1.1 g/Kg (by gastric tube) was the most successful, although marked variation still existed.

Rahman et al.15 have developed a model in male New Zealand white rabbits that were induced with 20methylolalanthen, glutathione depletion with busulphine sulfoxime, and subcutaneous administration of acetaminophen, which parallels clinical, biochemical, and histological features of human hepatic failure.

**Thioacetamide (TAA) model:** TAA-induced liver
failure has been studied for cerebral metabolic and histological effects.\textsuperscript{16} TAA has also been administered to rats in order to ascertain the role of astrocytes in HE.\textsuperscript{17} It was found that abnormalities in astrocytes, which precede any abnormal change in neurons, play a role in the development of HE. Celik \textit{et al}.\textsuperscript{18} have used combination treatment of HE with benzodiazepines and opioid-receptor antagonists. Chu \textit{et al}.\textsuperscript{19} recently compared the neurobehavioral scores and motor activities in normal, saline-treated and TAA-treated rats. A significant correlation was observed between these scores. This model in rats appears to be acceptable for study of pathogenesis and treatment of HE. Dimethyl sulfoxide and dimethylthiourea have also been found to protect against TAA-induced HE in rats.\textsuperscript{20}

\textit{Carbon tetrachloride model:} Animal models using this toxin have been developed by a few groups. An old study by Leenhoff \textit{et al}.\textsuperscript{21} showed liver cell necrosis in pigs.

\textit{Azooxy methane (AOM)-induced hepatic failure:} AOM injection in C57BL/6J mice was found to cause microvesicular steatosis, sinusoidal dilatation, centrilobular necrosis and mitochondrial injury. Late encephalopathy was associated with increased arterial ammonia, decreased serum glucose, and astrocyte swelling.\textsuperscript{22} The advantage of this new model was its reproducibility, its low-to-low variability, and dose-dependence. AOM thus emerges as an excellent agent for study of FHF.

\textit{Other models:} Hayase \textit{et al}.\textsuperscript{23} have suggested a cocaine-induced model where neurobehavioral and biochemical changes have been observed in mice.

\textbf{Conclusion}

Many approaches have been used to develop animal models of HE, each with its advantages and disadvantages. Specific requirements of studies on a specific aspect of HE also influence the choice of animal model. Astrocytes have been used in studies on cerebral edema,\textsuperscript{17} whereas Olafsson's model\textsuperscript{18} was designed to study brain edema and intracranial hypertension in rats. Surgical models lack the inflammatory mediators produced by damaged and/or necrotic hepatic cells.

Drug models have their own difficulties, such as reproducibility and extrahepatic toxicity, the cost of drugs and lack of a clinical syndrome of human equivalence. The clinical and biochemical criteria often bear little resemblance to those used in clinical practice. Animal-to-animal variation leads to lack of reproducibility. Drug dosage also plays an important role in HE. A good model should have a defined mortality rate.

\textbf{References}


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