A surgical model of fulminant hepatic failure in rabbits

Kuo-Chen Hung1,5, Chee-Chien Yong1, Yaw-Sen Chen1,5, Hock-Liew Eng2, Fang-Ying Kuo2, Chi-Chang Lin1, Tai-Horng Young3, Eiji Kobayashi4, Chao-Long Chen1 and Chih-Chi Wang1

1 Department of Surgery, Chang Gung Memorial Hospital, Kaohsiung Medical Center, Kaohsiung Hsien, Chang Gung University College of Medicine, Taiwan
2 Department of Pathology, Chang Gung Memorial Hospital, Kaohsiung Medical Center, Kaohsiung Hsien, Chang Gung University College of Medicine, Taiwan
3 Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, Taipei, Taiwan
4 Division of Organ Replacement Research and Animal Transgenic Research Center for Molecular Medicine, JiChi Medical School, Tochigi, Japan
5 Department of Surgery, E-Da Hospital and I-Shou University, Kaohsiung, Taiwan

Keywords
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Abbreviation
FHF, fulminant hepatic failure; ICP, intracranial pressure; LMI, liver mass index

Correspondence
Chih-Chi Wang, MD, Department of Surgery, Chang Gung Memorial Hospital, Kaohsiung Medical Center, Chang Gung University College of Medicine, 123, Ta-Pei Road, Niao Sung Hsiang, Kaohsiung Hsien, Taiwan. Tel: +886 7 7311723, ext: 8093 Fax: 886 7 3790719 e-mail: ufel4996@ms26.hinet.net

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Abstract
Aim: Animal models of fulminant hepatic failure (FHF) have been developed for characterization of disease progression and to evaluate the effectiveness of liver-assist devices, some by treatment with hepatotoxic drugs, viral hepatitis or surgical procedures. We have developed a model in the rabbit by combining resection of the three anterior lobes with ligation of the pedicle of the right lateral lobes, resulting in liver necrosis; the remnant quadrate lobes are left intact.

Materials and methods: Adult male New Zealand white rabbits (n = 16) were used. Six animals were killed to measure the weight of the separate liver lobes. The others (n = 10) underwent left neck central line placement to monitor continuous blood pressure and collect blood for laboratory analysis, and a burr hole on the right parietal bone to monitor the intracranial pressure (ICP). Blood laboratory analysis, clinical hepatic encephalopathy and ICP levels were measured in FHF animals (n = 6). Animals (n = 4) undergoing a sham operation served as controls.

Results: All FHF animals died between 12 and 26 h after liver surgery from FHF characterized by a progressive increase in liver enzymes, ammonia, total bilirubin, coagulopathy, hepatic encephalopathy and intracranial hypertension. Histological features of the ischaemic lobes showed coagulative necrosis of hepatocytes with absence of nuclei and collapse of cell plates. Brain histology revealed hypoxic cell damage.

Conclusion: We have developed a simple, reproducible model of FHF in rabbits that has a number of features comparable with clinical FHF patients and is well suited for testing experimental bioartificial liver systems and investigating the pathogenesis of FHF.

Fulminant hepatic failure (FHF) is characterized by rapid onset of encephalopathy following jaundice. FHF is the result of rapid loss of hepatic functions, including metabolic and detoxification functions, precipitated by toxic assault on hepatocytes because of viral hepatitis, idiosyncratic responses or drug overdose. Although liver transplantation is the most accepted mode of treatment for FHF, it is hampered by shortage of donor organs and requires life-long immunosuppression (1). An alternative treatment is needed to bridge the gap until liver transplantation and to allow time for liver regeneration (2). This has led to the development of extracorporeal bioartificial liver devices for short-term support until a suitable organ becomes available (3–5).

The aetiology of FHF is complex and has led to numerous attempts to develop appropriate animal models to help in understanding the pathophysiology of FHF, and to explore various treatment options such as liver cell transplantation or bioartificial liver support (6, 7). Experimentally, FHF can be induced in animals either surgically, through viral hepatitis (8, 9) or by treatment with drugs such as D-galactosamine (10–13) or acetaminophen (14, 15). Both small animals, such as mice and rats (16, 17) and large animals, such as dogs and pigs (15, 16, 18, 19), have been used for laboratory models of FHF. Clinical characteristics, biochemical profiles or histopathological phenotypes are dependent on the method used for inducing FHF. Terblanche and Hickman (20) established the criteria...
for an ideal animal model of FHF and stated that an ideal animal model should display reversibility and reproducibility; it should lead to death from liver failure, but also provide a sufficient therapeutic window. Large animal models are preferred, and they should pose minimal hazard to personnel.

Surgical models of FHF have predominantly concentrated on devascularization by portocaval shunt or complete removal of the liver. Such models have biochemical profiles that poorly mimic the clinical conditions observed in humans. Further, these models are reversible only by transplantation and hepatic coma is short lived. Viral hepatitis infection is still a major cause of FHF in some areas of the world. A viral model has been developed recently by using rabbit haemorrhagic disease virus (RHDV) to induce acute liver failure (8, 9); this viral model could fulfill many criteria of a good model for FHF (21). Hepatotoxic drug models include induction of FHF by carbon tetrachloride, D-galactosamine and acetaminophen. These models induce rather well-defined pathological conditions, but they do not reproduce the same histopathological findings as clinical profiles in humans. Induction of FHF by combined hepatectomy and ischaemia in the liver was successfully produced in rats (18).

In order to address biocompatibility and physiologic metabolic function, primary human hepatocytes appear to be the natural choice for cell-based therapy in humans (22). Owing to the shortage of primary human hepatocytes, small- or moderate-scale experimental bioartificial liver systems are needed. We have developed a medium-size animal model to evaluate such systems in rabbits by combining resection of the three anterior liver lobes with ligation of the pedicle of the right lateral lobes; the clinical, biochemical, haemodynamic, intracranial pressure (ICP) and histological features of this model are described. The primary aim of this study was to develop a controlled, surgical model of acute liver failure with complete mortality in rabbits. The medium-size animal model is suitable for testing of a new liver support system. Resection of a large volume of liver and stay of ischaemic liver were relatively mimicking the clinical human setting. The application of liver support system to this animal model will be performed in continuous research.

Materials and methods

Animals

Adult male, New Zealand white (NZW) rabbits, weighing 2.1–2.9 kg, received water and pellet feed ad libitum and were kept in cages at 21 °C on a 12-h light/12-h dark cycle. All animal procedures were approved by the Animal Ethics Committee of Chang Gung University, based on guidelines for Laboratory Animal Facilities and Care of Chang Gung University.

The average weight of the liver and its individual lobes, including the three anterior lobes (left lateral, left medial and right medial liver lobes), right lateral lobes and quadrate lobes, were estimated by measuring the livers from six animals. FHF was induced surgically, as described below, in a further six animals and monitored at multiple times (preoperative, 6, 12 and 18 h post-surgery) for blood chemistry, clinical hepatic encephalopathy and ICP. Sham operations were performed on four animals as a control group for comparison.

Surgical procedure

Overnight-fasted animals were anaesthetized by an intramuscular injection of ketamine (50 mg/kg) and Rompum (1 ml/kg, Bayer Leverkusen, Germany). After general anaesthesia, each animal received an intravenous fluid infusion with normal saline (20 ml/kg) via a right ear marginal vein to prevent hypovolaemic status. Body temperature in FHF animals was maintained with lamps and heating pads. Rectal temperature was continuously monitored by an electronic probe (Philips V24E, M1205A, Boston, MA, USA). The sequence of the procedures was central line operation, induction of FHF and then the burr hole procedure.

Central line operation

Central line operation was initiated with a small skin incision on the left of the neck, with care taken to avoid bleeding. The left carotid artery was exposed and cannulated with a catheter (Browiac 4.2Fr, Bard, Salt Lake City, UT, USA) for continuous blood pressure monitoring (Philips) and blood sampling. Next, the left external jugular vein was cannulated and used for the infusion of intravenous fluid and drugs. Hydroxyethyl starch solution (HAES-steril 10%, Fresenius Kabi, Deutschland GmbH, Friedberg, Germany) was infused with 20 ml/kg before the abdominal surgery to prevent hypovolaemic shock owing to liver resection.

Induction of FHF

A midline incision of the abdomen was followed by dissection of the falciform and the left triangular ligament to expose the hepatic vein. The vascular structures and biliary tract (hepatic vein, portal vein, hepatic artery and hepatic duct) to the right lateral liver lobe were ligated. Then, the bilateral medial liver lobes were ligated together and resected, followed by a
similar procedure for the left lateral liver lobe. Special care was taken to fully mobilize the three anterior liver lobes and to place a ligature high around the common pedicle of the left lateral lobe, so that there was no interference with inflow and outflow of the remnant liver lobe (the quadrate lobe) (Fig. 1).

Burr-hole operation

Following the hepatic procedure, a burr hole (3 mm) was drilled over the right parietal skull bone and a dura mater incision (1 mm) was created. Baseline ICP was measured by inserting the tip of a Codman MicroSencor\textsuperscript{R} catheter (Codman MicroSencor, Johnson & Johnson, MA, USA) approximately 3 mm into the parenchyma of the right hemisphere. After initial measurement, the sensor was removed and the scalp wound was dressed with tetracycline ointment and closed with 3-0 nylon sutures.

Clinical monitoring and biochemical measurements

Clinical parameters were monitored postsurgery, including blood pressure and heart rate. Fluid supply with normal saline was maintained at 5 ml/kg/h. In the case of development of hypotension (mean blood pressure < 50 mmHg) or significant elevation of heart rate, fluid resuscitation with normal saline (20 ml/kg) was performed. After recovery from anaesthesia, the behaviour of the animal was monitored regularly. Encephalopathy scoring was adapted from that used by Traber et al. (19) Blood samples were taken pre-laparotomy, and at 6, 12 and 18 h after induction of FHF. Arterial blood gas, alanine transaminase (ALT), aspartate transaminase (AST), ammonia, total bilirubin, lactate dehydrogenase (LDH), prothrombin time (PT), sodium and potassium levels were measured using standard techniques. Blood sugar was examined every 4 h using a blood sugar machine (ONE TOUCH Profile, LIFESCAN, CA, USA). Hypoglycaemia was prevented by infusion with 2 ml of 50% glucose/water when the blood sugar level fell below 80 mg/dl. An ICP transducer was used to monitor continuously ICP in surgically treated animals after the development of severe hepatic encephalopathy (19).

A postmortem examination was performed immediately, for analysis of the macroscopic appearance of the abdominal organs and brain. Biopsy specimens of the liver and brain were obtained from each animal and observed under standard H&E staining according to established procedures.

Statistical analysis

Data are presented as mean ± SEM. Statistical significance was determined by a t test. Correlation was determined by Pearson’s product–moment correlation coefficient, \( r \). \( P \) values of \( \leq 0.05 \) were considered to be significant. Statistical analyses were performed using SPSS for Windows version 11.0 (SPSS, Chicago, IL, USA) and EXCEL 2000 (Microsoft).

Results

As a first step, the average liver weight and relative sizes of different liver lobes were estimated using a group of six healthy normal NZW rabbits. All rabbits tolerated the operations well, with minimal intraoperative blood loss. No surgical complications were detected during the period of observation in this study. The operative time of laparotomy, central line placement and burr-hole operation was less than 50 min. Control group animals recovered from anaesthesia about 2 h after surgery and showed no overt paralysis or muscular weakness. In the liver weight and control groups, the three anterior lobes were 74.12 ± 1.02% of liver mass; the right lateral lobe was 20.22 ± 0.95%; and the quadrate lobe was 5.67 ± 2.51%. The liver mass index (LMI) was the ratio of weight of lobes (g)/body weight (kg). The LMI of three anterior and right lateral lobes was 21.75 ± 1.49 and quadrate lobe, 1.31 ± 0.11. In
FHF animals, the LMI of three anterior and right lateral lobes was 24.30 ± 1.36 (P = 0.3) and quadrate lobe, 2.22 ± 0.32 (P = 0.002). Correlation between survival and LMI of quadrate lobes was significant (r = 0.889).

FHF was induced as described in a set of six animals, and onset of mortality was observed 12–26 h (19.3 ± 2.0) postsurgery (Fig. 2). FHF animals recovered from the anaesthesia 4 h postsurgery but with slow ambulatory motions. This was followed by prostration, impaired balance and side recumbency. Body temperature in FHF rabbits declined, and hence was maintained at 38.5–39.5 °C by passive warming (heating lamp and pads). Increased heart rate (> 270/min) unresponsive to fluid resuscitation and rapid respiration rate with mild distress developed. Consciousness level then decreased markedly with onset of hepatic encephalopathy grade III about 10 h postsurgery. Grade IV hepatic encephalopathy followed, and was short with rapid onset of mortality. Muscular twitching was consistently observed before death.

As shown in Fig. 3, AST, ALT and ammonia levels increased progressively in FHF animals; they remained steady and low in the control group. Hypoglycaemia developed within 6 h postinduction and hence blood sugar was maintained by infusion of dextrose solution. A significant increase in LDH activity and bilirubin as well as prolongation of PT was also observed. While blood electrolyte (Na⁺, K⁺) levels remained normal, mild acidosis was found in the postoperative period owing to mild CO₂ retention under the effect of anaesthesia. These biochemical studies clearly showed rapid deterioration of liver functions and onset of hepatic injury.

Elevation of ICP (P < 0.05 vs. baseline) occurred when hepatic encephalopathy grade III developed and a rapid increase in ICP was observed before death (Fig. 4). Episodic and short increases in ICP, involving sudden elevation, followed by a gradual decline in ICP, were also observed frequently.

Postmortem examination was performed on all animals to analyse the histopathological complications arising from FHF in this model. In FHF animals, the ischaemic right lateral lobes were scattered with diffuse haemorrhagic spots, while the residual omental lobe showed normal colour. The rest of the abdominal organs were macroscopically normal, with no sign of venous congestion in the spleen, bowel, and the mesentery. No evidence of bleeding around the transducer site or into the subdural space was observed. Under light microscopy, the ischaemic lobe in FHF animals showed focal, lobular coagulative necrosis of haepatocytes with absence of nuclei and collapse of the cell plates (Fig. 5); marked dilatation and congestion of central vein and sinusoids; microvesicular fatty change and dissociation of haepatocytes. Brain biopsy in FHF animals showed nuclear pyknosis of neurons with indistinguishable cytoplasm and neural death scattered over the cortex. Control animals displayed normal histology.

**Discussion**

FHF in humans is associated with a very high mortality rate and presents as the terminal effect of many types of liver injury. This study is an effort to develop an animal model of FHF for a better understanding of the mechanisms and aetiology of FHF in humans. A novel, stable, and reproducible surgical model of FHF in rabbits is described, which retains many features seen in FHF patients, including progression of hepatic encephalopathy, biochemical alterations and coagulopathy. The major role of this model of FHF in rabbit is to provide the clinician with a more controlled experimental environment that does not ordinarily exist in clinical practice. Although the early surgical models had many high variations in survivals, these more refined resection plus ischaemic models appear to mimic more closely the changes that occur in liver failure. More than with other models, there is no need to perform shunt between portal and systemic veins can be easily reproduced in surgeons.

The need for animal models of FHF is well documented, with numerous attempts using both small and large animals. FHF is usually induced in these animals either by feeding the animals with hepatotoxicants such as d-galactosamine (10–13) and acetaminophen (14, 15) or surgically by total liver resection/devascularization (23–26). The drawbacks of hepatotoxicant-based FHF induction include poor reproducibility of the model and complications caused.

![Fig. 2. Survival rate in fulminant hepatic failure animals.](image-url)
Fig. 3. Time courses of serum levels of aspartate transaminase (a), alanine transaminase (b), lactate dehydrogenase (c), total bilirubin (d), ammonia (e), prothrombin time (f) and pH (g) were measured postsurgery in both fulminant hepatic failure and control animals, indicating marked liver injury and loss of liver function.
by extrahepatic effects of these drugs (e.g. acetaminophen). D-galactosamine-induced FHF is particularly atypical in its clinical manifestation and hence forms a poor model for studying the human disease (20, 26).

In viral model, RHDV has been shown to be an adequate animal model for FHF in rabbit. However, the study was limited to rabbit, although it provided minimal risk of transmission to other vertebrate species (27).

Potential disadvantages with existing surgical models based on partial hepatectomy (28, 29), or total hepatectomy (23, 24) are their inability to recreate the inflammatory milieu that exists in acute liver failure. They are accompanied by a stress response that alters the underlying pathophysiological state of liver failure. Furthermore, their relevance as a cause of liver failure in humans such as viral hepatitis and drug-induced hepatotoxicity is rather limited. In this study, we combined the resection of the three anterior lobes (74.12% of the liver) with ligation of the pedicle of the right lateral lobes (20.22% of liver) while the remaining caudate lobes (5.67% of liver) were left intact. This modified version of the previously published procedure in rats (18) is advantageous because it allows for simple surgical induction of FHF displaying various

Fig. 4. Intracranial pressure in fulminant hepatic failure animals post surgery.

Fig. 5. Histopathological features of: (a) right lateral liver lobe of fulminant hepatic failure (FHF) animals, × 100, hematoxylin and eosin (H&E) staining; focal, lobular coagulative necrosis of hepatocytes with absence of nuclei and collapse of cell plates; (b) right lateral liver lobe of FHF animals, × 100, H&E staining; marked dilatation and congestion of central vein and sinusoids; (c) brain biopsy of FHF animals, × 40, H&E staining; neuronal death in the dentate gyrus of hippocampus; (d) brain biopsy of FHF animals, × 200, H&E staining; cell death in large clusters characterized by nuclear alteration (pyknosis). The nucleus is shrunken and stained darkly. The cytoplasm is indistinguishable.
degrees of severity of disease (based on the amount of native liver left intact in the animal). Ischaemic models of FHF (30, 31) involve complicated surgical procedures, resulting in a greater reliance on surgical expertise (26) and the potential for animal-to-animal variation dependent on the surgeon. Clinical progression of FHF in this surgical model was found to be very similar to that found in human patients, including rapid onset of grade III/IV hepatic encephalopathy, and death between 12 and 26 h postsurgery. Biochemical analysis clearly indicated signs of progressive liver failure and coma, and the animals had a mean survival time of 19.3 h, which may be sufficient for testing support procedures such as treatment with a bioartificial liver. No significant abnormalities in blood electrolytes or blood pH were observed. Histopathological analysis clearly indicated massive hepatic necrosis, and was reflected in elevated plasma levels of AST and ALT.

All animals recovered from anaesthesia and remained haemodynamically stable with continuous infusion of saline. However, decompensated severe hypotension, unresponsive to fluid resuscitation, developed at the end of the experimental period. Arterial O2 saturation was around 98%. PaO2 was maintained at more than 100 mmHg in room air.

Brain oedema is a major cause of death in FHF patients (32). The pathogenesis of brain edema and intracranial hypertension in FHF is not clear. Three hypotheses have been proposed: (1) the accumulation of glutamine within astrocytes of the cerebral cortex, (2) products arising from the necrotic liver and (3) abnormalities of the cerebral circulation. The heterogeneous presentation of ICP change in this study implied that multiple factors were involved in the pathogenesis of FHF. ICP was elevated in animals with severe haepaticoencephalopathy (19) and we applied the ICP transducer when grade III hepatic encephalopathy developed. Continuous monitoring until death demonstrated elevated ICP in all animals but there were differences in the degree and pattern of ICP changes. The histology of the brain biopsy in FHF animals revealed cell death in large clusters characterized by pyknosis and indistinguishable cytoplasm. It was comparable with hypoxic neuron damage, whereas systemic arterial blood gas did not show low oxygen saturation during the experiment.

In our preliminary study (data not presented), FHF rabbits (n = 6) maintained at ambient (21 °C) temperature developed mild hypothermia (32–34 °C) and had a longer survival time (35.5 h) than normothermic FHF animals at 39 °C (19.3 h). It has been reported previously that mild hypothermia has a beneficial effect on uncontrolled brain oedema in FHF (33, 34). One hypothesis for this observation is that hypothermia decreases metabolic demands and reduces the neurotoxicity of ammonia (18).

In the postmortem examination of the FHF animals, there was no significant sign of venous congestion in the spleen, bowel and mesentery, suggesting portal hypertension. There was only a modest (20–22 cmH2O) increase in portal pressure with acute constriction of the portal vein to < 20% of the cross-sectional area (18). The mass of the quadrate lobe was statistically different between FHF and control animals (LMI, P = 0.002). The increase in residual normal liver weight was caused by oedema (18). Interestingly, there was an association between survival time and LMI of the quadrate lobe (r = 0.889). Hence, there is the possibility of a longer survival time due to larger liver mass (oedema) or larger intake liver (not the oedema portion) resulting in extended survival.

The amount of haepatocytes to be loaded in a bioartificial liver for effective support of FHF patients is a fundamental design criterion that has remained unclear. This drawback may have contributed to the inability of many clinical efforts to show efficacy in a statistically significant manner (35). The difficulty in estimating the extent of normal liver function in FHF patients has led to the construction of a standardized BAL therapeutic mode that may not be able to recompense liver functions sufficiently in severely affected patients. These factors have not been studied in detail in most animal models, as developing a reliable FHF model with various degrees of severity has been very difficult. The current surgical procedure, which allows for control of the extent of normal liver function, allows for exactly these kinds of studies. There was < 10% viable liver mass left in the quadrate lobe. This is a potential reversible model if there is an effective treatment bridging the FHF animal to the stage of sufficient regenerated liver mass. The extent of liver injury should be measurable in an ideal animal model of FHF (18). The residual normal liver function is potentially predicted in this model owing to the advantage of the rabbit liver anatomy, which is separated into five liver lobes.

We have successfully combined two surgical procedures (haepatectomy and ligation of the pedicle of the right lateral lobe), resulting in a reliable, potentially reversible model of FHF in rabbits that displays a number of clinical, biochemical and histological features comparable with clinical FHF patients. This novel medium-size animal model is simple and reproducible and well suited for testing bioartificial liver support procedures such as treatment with a bioartificial liver.
support in FHF and the study of more detailed pathophysiology of FHF.

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