An Improved Model of Acute Liver Failure Based on Transient Ischemia of the Liver

Inge Fourneau, MD; Jacques Pirenne, MD, PhD; Tania Roskams, MD, PhD; Sing-Hiem Yap, MD, PhD

Hypothesis: A reproducible and potentially reversible model of acute liver failure in the pig is feasible based on transient ischemia of the liver.

Design: To determine the shortest period of liver ischemia sufficient to cause 100% mortality, ischemia of the liver was induced for different lengths of time, starting with 6 hours. If the pig survived, ischemia time was prolonged for 2 hours in the next animal. In the first group, the common bile duct was not tightened. In the second group, the common bile duct was tightened.

Setting: The Laboratory for Hepatopathophysiology, Catholic University, Leuven, Belgium.

Participants: Female stress-negative Belgian Landrace pigs weighing 18 to 22 kg.

Interventions: During preparatory surgery, all ligaments around the liver and connective tissue around the liver hilum were transected and an end-to-side portacaval shunt was made. Vessel loops were placed around the branches of the hepatic artery and bile duct. Three days later, in fully awake pigs, the loops were tightened.

Main Outcome Measures: Mortality. Development of acute liver failure was determined based on neurologic, biochemical, and pathological variables.

Results: When occluded for 10 hours, 5 of 6 pigs in group 2 died between 12 and 17 hours after the induction of ischemia. All pigs developed typical acute liver failure. Tissue specimens showed 90% necrosis of the liver parenchyma.

Conclusion: A highly reproducible and potentially reversible model of acute liver failure in the large animal has been established.

Arch Surg. 2000;135:1183-1189

Despite intensive medical treatment, the mortality of acute liver failure (ALF) is high. With the introduction of orthotopic liver transplantation, 1-year survival increased to 80%. However, liver transplantation is hampered by a severe shortage of donor organs. This limited availability of donor organs has led to a new impetus for the development of liver-supporting systems, ie, bioartificial liver, to prevent the patient from dying while waiting for liver transplantation or even to avoid transplantation if regeneration can be secured. Although various bioartificial liver-supporting systems have already been introduced in clinical trials, evaluation of the safety, efficacy, and mechanism of action of liver-assist devices would be greatly facilitated if they could be tested in a clinically relevant large animal model. Extensive research has been conducted to develop a suitable animal model of ALF. Terblanche et al evaluated the various animal models of ALF in 1974 and updated their findings 15 years later. According to their findings, 6 criteria are required for a “satisfactory” animal model: reversibility, reproducibility, death from liver failure, sufficient therapeutic window, large animal model, and minimal hazard to personnel. We believe that a seventh requirement should be added: liver failure should be induced in a conscious animal without influence of anesthetics or sedation to be able to evaluate the development of encephalopathy, since it is an essential part of the clinical features of ALF.
PARTICIPANTS AND METHODS

Female stress-negative Belgian Landrace pigs weighing 18 to 22 kg were used. The study protocol was reviewed and approved by the Research Animal Resources and Animal Care Committee of the University of Leuven, Leuven, Belgium.

ANESTHESIA AND RECOVERY

The animals were premedicated with azaperone (Stresnil) intramuscularly. General anesthesia was induced by an initial intravenous injection of propofol (1% Diprivan) and ketamine hydrochloride (Imalgene). Animals were intubated with a cuffed endotracheal tube and connected to an anesthesia ventilator (Engström 300; Engström, Solan, Sweden). Anesthesia was maintained with a mixture of oxygen and nitrous oxide and continuous administration of propofol. At the beginning of surgery, 300 mg of ampicillin (Penbritin), 80 mg of gentamicin sulfate (Geomycine), and 50 mg of ranitidine (Zantac) were administered intravenously. After discontinuation of anesthesia, the animals were kept on the operating table until they were awake and breathing adequately. Animals were extubated after transport to the cage. This cage, equipped with a 3-channel swivel-tethering system, allowed comfortable maintenance of fully ambulatory pigs while ensuring continuous blood pressure monitoring and administration of 5% dextrose and 0.9% sodium chloride, both at a rate of 1 L/24 h. For the prevention of stress ulcers, 50 mg of ranitidine was administered intravenously twice a day.

PREPARATIVE SURGERY

On day 0, a single-lumen catheter was placed into the common carotid artery for continuous blood pressure monitoring and blood sampling. A double-lumen catheter was inserted into the external jugular vein for the administration of intravenous fluids. Therefore, the skin was incised for 10 cm at the anterior margin of the sternocleidomastoid muscle. The external jugular vein and the common carotid artery were then exposed at the anterior and posterior sides of the sternocleidomastoid muscle, respectively, and cannulation was performed. The catheters were tunneled and brought up to the dorsal part of the neck.

On day 3, a xyphopubic laparotomy was performed. The bladder was opened, and a Foley catheter was inserted to monitor the urine production. The liver was skeletonized, excising all its connecting ligaments. All structures in the hepatoduodenal ligament, except the portal vein, the hepatic artery, and the common bile duct, were divided. The common bile duct and the common hepatic artery together with the inbranching gastroduodenal artery were isolated, and a vessel loop was placed around the proper hepatic artery just at its bifurcation into its right and left liver branch. A second vessel loop was placed around the common bile duct. The right gastric artery was also surrounded with a vessel loop under all circumstances to prevent backflow. Depending on the anatomic features, in some of the animals the gastroduodenal artery was also surrounded. An ultrasonic perivascular blood flow sensor (Transonic Flowprobe; Transonic Systems, Ithaca, NY) was positioned around the isolated hepatic artery. The loops were only proof tightened, the point of complete vessel obstruction marked by a node at the external abdominal wall. The loops were passed through the abdominal wall and fixed with a pin. The infrahepatic inferior vena cava was dissected beginning from its insertion into the liver parenchyma down to the inlet of both renal veins. Afterward, the portal vein was dissected free up to the splenic vein. In some animals, a pancreatic vein needed to be ligated and transected. The inferior vena cava and the portal vein were longitudinally clamped and a 3-cm side-to-side portacaval shunt was performed, followed by ligation of the portal vein close to the liver hilum to create a functional end-to-side shunt.

In 1985, de Groot et al² developed a reversible model of ALF based on 6 hours of transient liver ischemia in the pig. Although almost completely fulfilling all requirements for a satisfactory animal model, a basic disadvantage of this model was its lack of reproducibility as expressed in a nonuniversal mortality and a wide range of survival time.

In this report, we present a highly reproducible model of ALF in the pig, fulfilling all previously mentioned criteria.

RESULTS

SURVIVAL

The outcome of animals in the different protocol groups is illustrated in Table 2. In group 1, even after 14 hours of occlusion of the hepatic artery alone, no universal mortality was obtained. In group 2, 5 of 6 animals subjected to 10 hours of occlusion of the hepatic artery and the common bile duct died within 17 hours after the induction of ischemia. In all pigs, death was due to hepatic coma. In the ensuing report, the neurologic, clinical, biochemical, and histological features of this second group will be described more extensively.

NEUROLOGIC ASSESSMENT

All animals followed a uniform course. They woke up spontaneously when the anesthesia was interrupted. In the 72 hours following portacaval shunt, they developed decreasing spontaneous activity. The evolution of the encephalopathy score after the induction of ischemia is presented in Table 3. After 7 to 10 hours of ischemia, the animals developed a cerebellar gait and impaired balance. Soon after liver arterial reperfusion, they developed increasing restlessness, followed by sopor. Consciousness decreased markedly. Finally, they became comatose, accompanied by muscular twitching and later rigidity. Terminally, there was gasping with cyanosis and convulsions.
Figure 1). The wound was then closed. Throughout the surgical procedure, approximately 1 L of 0.9% sodium chloride and 1 L of 5% dextrose were administered.

INDUCTION OF ISCHEMIA

On day 6, 72 hours after a portacaval shunt was performed, the vessel loops around the hepatic artery, the right gastric artery, and the gastroduodenal artery were tightened. Arrest of the hepatic blood flow was confirmed by total disappearance of the signal obtained by the blood flow sensor. During this procedure, the animals remained quiet. In a pilot study, the minimal ischemia time necessary to obtain a universal mortality was determined. For this purpose, the loops were tightened for increasing duration, starting with 6 hours. Afterward, the vessel loops were cut and pulled off. Restoration of blood flow was confirmed by continuous monitoring by the ultrasonic blood flow sensor. If the pig survived, ischemia time was prolonged for 2 hours in the next animal. In the first series (group 1), the loop around the common bile duct was not tightened. After 14 hours of occlusion of the hepatic artery alone, still no universal mortality could be obtained; the vessel loop around the common bile duct was tightened in the animals in group 2.

Thirty minutes before tightening and releasing of the vessel loops, 2 g of piperacillin sodium (Tazocin) was administered intravenously to prevent bacteremia due to cholangitis lenta.

CLINICAL OBSERVATIONS

After the induction of ischemia, the behavior of the animals was examined regularly. To evaluate more carefully the grade of encephalopathy, a scoring system was developed (Table 1). In addition to a general observation of an animal’s behavior, it was registered if the animals were able to walk or to drink and whether the eyes were opened spontaneously or only on acoustic or painful stimuli.

VITAL VARIABLES

The heart rate and systemic blood pressure remained fairly constant during the experiment. No hypotension or tachycardia could be detected until just before death. Hyperventilation was noted starting a few hours before death. No hypothermia was noted. Urine output remained fairly constant.

BIOCHEMICAL MEASUREMENTS

Pigs showed 2 decreases in pH, a first 1 hour after the induction of ischemia (P = .09) and a second 1 hour after starting reperfusion (P = .03). The levels of plasma glucose, potassium, chloride, and sodium remained within the normal range. The levels of blood urea and creatinine did not change. In contrast, the levels of aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase (Figure 2) increased significantly until 1 hour of reperfusion. Subsequently, levels of alanine aminotransferase decreased, whereas levels of aspartate aminotransferase and lactate dehydrogenase remained unchanged. Levels of alkaline phosphatase (Figure 3) remained increased until 3 hours of reperfusion (P = .03). Levels of blood ammonia (Figure 4) increased until 1 hour after reperfusion before reaching a steady state. Levels of γ-glutamyltransferase (P = .33) and blood lactate (P = .22) did not change. Levels of total bilirubin (Figure 5) increased slowly with increasing occlusion time, followed by a significant decrease after reperfusion. Levels of coagulation factors (factors V and X) decreased with increasing ischemia time (Figure 6), as did levels of fibrinogen (P = .04) (Figure 7). A significant decrease of levels of partial thromboplastin time (Figure 6) was seen during ischemia, followed by a steady state. No significant effect was observed on activated partial thromboplastin time (P = .36).

HISTOLOGICAL CHARACTERISTICS

The baseline biopsy specimen showed normal liver tissue without signs of preexisting liver disease. On the
biopsy specimen taken immediately after performing the portacaval shunt, signs of only minimal surgical necrosis were seen. A postmortem examination was performed in all animals. Less than 200 mL of fluid was found in the abdomen. The liver was spotted, the color varying from purple to pale. The rest of the abdominal organs were macroscopically normal. There was no sign of venous congestion in the mesenteric area. The portacaval shunt, the arterial vessels, and the common bile duct appeared patent. After opening the stomach, no gastric bleeding could be found. The results of a microscopic examination of the postmortem liver specimens revealed nearly complete coagulation necrosis of the liver parenchyma. Periportal, there was only a thin border of persisting viable hepatocytes (Figure 8).

**COMMENT**

There is some difficulty in evaluating new treatment modalities for ALF by means of controlled clinical trials because of the high mortality rate of that condition, the multiplicity of factors affecting outcome, and the excellent results provided by orthotopic liver transplantation. As a critically ill patient cannot be denied a liver transplantation, which is probably the only chance for survival, it will be extremely difficult to demonstrate the efficacy of the bioartificial liver as a bridge to regeneration in ALF by clinical trials only. Prior testing in a clinically relevant large animal model is, therefore, more appropriate and a prerequisite.
The available models for the treatment of ALF include surgical anhepatic and devascularization procedures and hepatotoxic drug administration. Combined surgical and drug models have also been reported. However, previous studies have failed to develop a model fulfilling all criteria, as proposed by Terblanche et al. Hepatotoxic drug models have several disadvantages. First, in most models, there is a lack of reproducibility, unpredictable outcome and dose response, and uncertain time of death after injury. Second, the toxic effect is not limited to the liver alone and the influence of a tested therapy can probably be biased by those extrahepatic effects. Surgical models such as hepatectomy or a portacaval shunt with irreversible ligation of the hepatic artery are not ideal because they lack potential reversibility and because of the release of potentially toxic products from damaged liver tissue into the circulation. To overcome these problems, Fischer et al created a model of reversible hepatic ischemia in ambulant conscious pigs. Ischemia was induced 24 to 48 hours after performing a side-to-side portacaval shunt by tightening a nylon sling around the hepatic artery and the liver side of the portal vein for 40 to 160 minutes. The minimum duration of hepatic ischemia required to produce death due to hepatic coma could not be estimated. Hepatic encephalopathy could not be induced in these studies, since the animals either survived or died without a clear period of clinically manifest neurologic abnormalities. Another major problem with this model is the risk of portal vein rupture. Therefore, this model was modified by de Groot et al. Three days after the creation of a functional end-to-side portacaval shunt and stenting of the common bile duct, 15 ambulant animals underwent total liver ischemia for 4 or 6 hours by closure of a mechanical clamp surrounding the hepatic artery. All but 1 of the animals undergoing 6 hours of hepatic ischemia developed grade 4 encephalopathy after 24 to 30 hours and died within 50 hours. The main problem with this model is the wide range of survival time. Therapeutic methods that may confer some benefit require models with narrowly delineated limits. For this reason, we modified the model further to obtain a more reproducible model with constant mortality.

A factor that could affect the tolerance of liver ischemia is the amount of toxins released after reper-

Figure 2. Mean ± SD levels of analytes after the induction of ischemia. A, Alanine aminotransferase (ALT). B, Aspartate aminotransferase (AST). C, Lactate dehydrogenase (LDH).

Figure 3. Mean ± SD levels of serum alkaline phosphatase after the induction of ischemia.

Figure 4. Mean ± SD levels of blood ammonia after the induction of ischemia.

Figure 5. Mean ± SD levels of serum total bilirubin after the induction of ischemia.
fusion of the liver. If one wants to enhance the reproducibility and to narrow the range of survival time, it is important that animals have similar gastrointestinal tract contents at the start of the experiment. Therefore, extensive cleansing of the bowel is essential. In an attempt to reduce the disturbances to the internal milieu, it is better to use preparative fluid therapy and fasting rather than preoperative laxatives. By doing so, the portacaval shunt can be performed in an animal with a well-equilibrated fluid balance, which reduces the risks of the surgical procedure. For this reason, we inserted infusion catheters during a small separate anesthesia 3 days before the portacaval shunt was performed. Two agents, 5% dextrose and 0.9% sodium chloride, were administered intravenously, both at a rate of 1 L/24 h. No solid food was given.

That the collateral circulation in the bile duct wall can provide an important blood flow to the liver is once again demonstrated in this study. Ischemia based on occlusion of the hepatic artery alone was not able to induce sufficient necrosis to reach consistent mortality. For this reason, other groups have inserted a silicone tubing into the bile duct with ligation of the wall on this tubing.4 However, insertion of a tube in the bile duct with subsequent ligation of the bile duct wall could be responsible for some permanent ischemia from the time of portacaval shunt on.

In the model described by de Groot et al,4 an ischemia time of 6 hours was recommended. We had to increase the ischemia time to 10 hours. This can possibly be ascribed to the use of smaller pigs. Moreover, insertion of a tube in the bile duct with subsequent ligation of the bile duct wall could be responsible for some permanent ischemia from the time of portacaval shunt on.

Does this model fulfill all requirements for a satisfactory animal model of ALF? As the arterial blood flow and the flow through the common bile duct are restored after release of the vessel loops, the potential for reversibility is present. In this group of animals, we achieved consistent mortality, with a range of survival time between 12 and 17 hours, which is an important improvement of the reproducibility compared with previous models. In all pigs, death was the result of hepatic coma, with a survival time long enough to allow hepatic support procedures to be evaluated. The model is performed in a large animal without biohazard.

Therefore, we can conclude that we describe herein a large animal model of ALF that is highly useful for the evaluation of the further development of liver-supporting systems. Experiments are in progress using this model to evaluate the performance of a new bioartificial liver.

We thank Tina Crabbé for technical assistance and Frank Van Gelder for the statistical analysis of the data and the illustration on page 1186.

Figure 6. Mean±SD levels of variables after the induction of ischemia. A, Factor V. B, Factor X. C, Partial thromboplastin time (PTT).

Figure 7. Mean±SD levels of fibrinogen after the induction of ischemia.

Figure 8. A postmortem liver specimen showing extensive coagulation necrosis. Only a small rim of viable hepatocytes is present (arrowheads) (hematoxylin-eosin, original magnification ×100).
Acute liver failure caused by fulminant hepatitis has at least 2 components—severe impairment or absence of liver function, and the effects of tissue destruction. The former effect can be simulated by rendering animals anhepatic. With adequate restoration of vascular connections, such animals will awake from anesthesia and appear normal for up to 60 hours. Even terminally, such animals seldom show features of hepatic encephalopathy, but rather of respiratory failure. This model is reasonably easy to reproduce; not so the attempts to use toxins or ischemia to simulate a human disease that probably occurs with recurrent waves of destruction compounding damage.

For more than 40 years, attempts have been made to simulate human acute liver failure to test forms of treatment, including extracorporeal liver perfusion in the 1970s and liver transplantation or the bioartificial liver in the 1980s and 1990s. The report by Fourneau et al adds to previous models the need for preoperative fluid and fasting, rather than purgative preparation of the bowels and temporary occlusion of the bile duct to ensure absolute ischemia to the liver. Such occlusion reduced survival to 1 of 6 animals, compared with 5 of 8 animals when there was no occlusion. But there is still that 1 animal among 6 statistic that almost certainly would recur if larger numbers of animals were prepared. Such a survival rate inevitably skews results among the small numbers that have to be used for large animal experiments.

An important contribution of this article is the description of the encephalopathy score. Reports of true encephalopathy in pigs are few, and this scoring system will lead to more uniform comparisons. Thus, although this article makes a useful contribution, and all attempts at creating models of hepatic failure must be documented to avoid repetition, the search for a more predictable and reproducible model without outliers must continue. Perhaps a scheme using repeated episodes of ischemia or repeated doses of toxin would be successful.

Rosemary Hickman, MD
Cape Town, South Africa
to need to be reserved for certain selected cases. The Indiana University group in Indianapolis has really published some very
thoughtful work on their complications, and they have had a
35% bar disruption rate that they noted with their early cases.
These have been corrected with some modifications of the bar,
but it is a fairly substantial complication rate.

Since the older patients are the ones who really have the
most pain, is there an age at which you will not recommend
using this technique?

Nuss recommends 24 months for the bars to be in. Most
of us who use other types of chest wall supports for pectus
excavatum repairs leave them in for about 6 months. Do you al-
low children who have these struts in place to play contact sports
while the bar is in place?

Dr Wu: I will begin first by addressing issues concerning
the physiologic effects and exercise intolerance related to pec-
tus excavatum [PE] deformities. These are major areas of study
and debate when evaluating surgical repair of PE defects. De-
spite numerous published studies on the physiologic effects
and measurements of physiologic parameters in PE deformity, there
has been no significant correlation established between the se-
vurity of PE deformity and the level of exercise intolerance. How
this applies to evaluating patients with severe defects is dif-
cult to determine. In a recent study appearing in the Journal of
Pediatrics, 13 patients with severe PE deformities were eval-
uated for exercise intolerance. This study concluded that dimin-
ished exercise intolerance seemed to be greatest in patients ex-
ercising in the supine position, and patients were least affected
by exercise in the prone position.

This may be an explanation for the apparent minimal im-
 pact of PE in an Olympic-class swimmer reported by Dr Cook.
Additionally, studies reported in the cardiothoracic literature
have consistently reported on improved cardiopulmonary func-
tion following surgical repair of PE.

The role of minimally invasive repair [MIR] in older pa-
tients is challenging. Our oldest patient undergoing MIR was
17 years of age. We have observed among our patients under-
going MIR, that the older patients have higher postoperative
analogic requirements and resultant longer hospital stays. The
data was skewed by several of the older patients requiring ex-
tended hospital stays reaching 12 days for pain management
issues. In fact, the majority of patients were able to be dis-
charged in 4 to 5 days, similar to patients undergoing tradi-
tional Ravitch repair. We currently utilize a standardized post-
operative pain management protocol incorporating epidural
analogesia, conversion to parenteral PCA [patient-controlled an-
alogesia] combined with oral narcotics and nonsteroids. De-
spite longer hospital stays in our older patients, these pa-
tients were among the most satisfied with their postoperative
results. This is an important point since the psychosocial im-
 pact of PE deformities cannot be overemphasized. Also, many
of the older patients had broad defects requiring the place-
ment of multiple bars to correct the deformity. Complications
of bar disruption have been drastically reduced with the rou-
tine use of lateral stabilization footplates to secure bar posi-
tioning. To avoid the risk of bar displacement, we advise our
patients to avoid contact sports prior to bar removal.

To address the issue of whether MIR is a better tolerated
procedure, I point out that other parameters, including shorter
operative times, minimal blood loss, and a short learning curve
make this procedure an acceptable alternative to traditional
costosternoplasty-type repairs. We have certainly observed
in our practice an increase in patient referral from our pediatric
community with the introduction of this minimally invasive
 technique.

We do not routinely use preoperative CT imaging and in-
dex scoring since we have found no definitive correlation be-
tween the severity of PE defect and physiologic impairment.
We perform MIR with thoracoscopic assistance in those pa-
tients with severe defects and mediastinal shift to avoid dras-
tic complications such as cardiac laceration.

There has been a major complication reported with the
Ravitch repair. There have been no cases of asphyxiating tho-
racic dystrophy reported by the original Nuss series or by oth-
ers, that have been previously associated with costosternop-
lasty repair. It is postulated that the more pliant hyaline cartilage
is replaced by stiff fibrocartilage. The MIR technique may avoid
this complication by eliminating the need for cartilage incision.

Currently, over 60% of polled pediatric surgeons in this
country utilize minimally invasive repair in patients with pec-
tus excavatum deformities. This attests to the overwhelming
support for a new and less invasive procedure introduced less
than 2 years ago by Dr Nuss for treatment of this challenging
pediatric disease.

Correction

Error in Text. In the Original Article by Fourneau et al titled “An Improved Model of Acute Liver Failure Based on Tran-
sient Ischemia of the Liver,” published in the October issue of the ARCHIVES (2000;135:1183-1189), errors occurred in the
abstract and the text. On page 1183, in the “Results” section of the abstract, the first sentence should have read as follows:
“When occluded for 10 hours, all pigs in group 2 (n=5) died between 12 and 17 hours after the induction of ischemia.” On
page 1184, in the “Results” section, under “Survival,” the third sentence should have read as follows: “In group 2, all animals
(n=5) subjected to 10 hours of occlusion of the hepatic artery and the common bile duct died within 17 hours after the
induction of ischemia.” The journal regrets these errors.