Editorial

**SIGNIFICANT ADVANCES TOWARDS A REPRODUCIBLE, CLINICALLY RELEVANT LARGE ANIMAL MODEL OF ACETAMINOPHEN-INDUCED ACUTE LIVER FAILURE**

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Acute liver failure (ALF) remains a devastating, life-threatening clinical problem that is defined as sudden hepatocellular necrosis in the absence of pre-existing liver disease. The onset and clinical course of ALF is unpredictable, and despite improved critical care management, mortality remains unacceptably high. Currently, to date, liver transplantation is the only curative treatment. However, owing to the rapid progression of ALF-induced multi-organ failure, and the persistent shortage of available organs, liver transplantation is very limited. Therefore, treatment options to bridge to liver transplantation or to spontaneous liver regeneration continue to be an unmet clinical need.

A major deterrent to the development of new therapies lies in the lack of a clinically relevant, reliable and reproducible animal model of ALF. Moreover, the diverse aetiologies of ALF, complex pathogenic mechanisms and multiple organ failure render it extremely difficult to establish a gold-standard model of ALF. In developing a clinically relevant animal model of ALF, the following important criteria are required: 1) Death caused by liver failure: this is pertinent to toxin models where extrahepatic toxicity becomes a confounding factor. 2) Reproducibility: predictable time to mortality and similar cause of death. 3) Reversibility: critical when investigating novel therapeutic strategies. In addition, a broad therapeutic window is valuable. Finally, given the advances in liver support systems, the use of a large animal model is beneficial to mimic clinical intensive care settings.

Acetaminophen is the most common cause of ALF (~40%) in the USA and Europe (particularly in the UK) [1]. Therefore, the creation of a large animal model of acetaminophen-induced ALF is clinically important and highly warranted. For close to over half a century, an enormous amount of research has gone into developing such a model; however, a major factor hindering the approval and acceptance of this toxin model has been the lack of reproducibility and inconsistent causes of death [2-4]. It has been demonstrated that the concentration of acetaminophen in the blood dictates the prognosis. Therefore, close monitoring of blood acetaminophen levels is essential in reducing extrahepatic toxicity, and for preventing fatal methaemoglobinemia. Pretreating animals with phenobarbital to induce cytochrome P450 liver enzymes [5] or buthionine sulfoximine (irreversibly inactivates glutathione synthetase to deplete liver glutathione stores) [6] are strategies to shorten the time to ALF and death. However, aside from the adverse effects, these pretreatment protocols weaken the clinical relevance of the model, and obscure the natural course and pathogenesis of acetaminophen-induced ALF.

We read with great interest the study by Lee et al., published in the November issue of *Liver International* (7). In that study, six pigs were administered acetaminophen, and the blood levels of acetaminophen were closely monitored (every 1–4 h). After an initial bolus injection, acetaminophen was continuously administered and the dose was thereafter titrated to maintain plasma levels of methaemoglobin between 1–5%. If methaemoglobin was <1% and accompanied by no relative increase in prothrombin time (PT), the acetaminophen dose was increased. Once ‘irreversible’ ALF was achieved (defined as PT > 60 s), acetaminophen administration was discontinued. All six pigs attained ‘irreversible’ ALF by 19.3 ± 1.8 h, and thereafter survived for a further 12.6 ± 2.7 h.

In the end, all six acetaminophen-induced ALF pigs died of intracranial hypertension within ± 2.7 h (coefficient of variance: 0.226). This thus defines, for the first time, a large animal model of acetaminophen-induced ALF that reliably predicts the

time to mortality and the cause of death. Furthermore, all animals developed multi-organ failure including severe circulatory disturbances including a high-output low resistance state which was closely monitored and managed according to intensive care standards with volume and large doses of vasoactive medication. In addition, these animals were placed on continuous renal replacement therapy to control electrolyte and acid base disturbances. These specific interventions and managing strategies prolonged the lives of these animals. However, each of these interventions potentially interferes with the pathophysiologic response and prognosis in ALF. Furthermore, whether ‘irreversible’ ALF – as termed in this study – is truly past ‘the point of no return’ requires further evaluation and confirmation. The King’s College Hospital selection criteria for liver transplantation for acetaminophen-induced ALF is pH < 7.30 after resuscitation or INR>6.5, creatinine >3.4 mg/dL and stage III or IV hepatic encephalopathy (HE). In the study by Lee and colleagues, ‘irreversible’ ALF was defined when PT > 60 s. However, at this time point, although intracranial pressure (ICP) was not significantly increased, renal failure progressed despite continuous renal haemodialysis. Therefore, the definition of ‘irreversible’ ALF in this model merits to be evaluated. This is important as liver recovery is a vital rescue option for patients with acetaminophen-induced ALF; spontaneous liver regeneration occurs in close to 60% of these patients [1]. Furthermore, defining ALF as ‘irreversible’ evidently implies that this proposed model is not reversible; an essential criteria for the development of an animal model of ALF. Therefore, whereas this model of intensive care management is not designed to study basic pathophysiologic mechanisms, it may instead serve as an excellent model to study specific treatment protocols.

Given that intracranial hypertension developed in all six acetaminophen-induced ALF pigs, this undeniably illustrates the presence of brain oedema. The development of hepatic encephalopathy is a cardinal feature of ALF and in anaesthetized animal models ICP is the sole reliable marker of neurological decline. Osmotic changes in the brain typically target the astrocytes in HE, affecting cerebral function. In addition, the physical presence of brain oedema impacts on ICP causing brain stem herniation (appearance of Cushing reflex). However, the neuropathology and neurobiology remain to be fully characterized in the proposed model.

In addition, the results of this study demonstrate that the surge in ICP is accompanied by a rise in blood ammonia, confirming a tight relationship between these two factors [8, 9]. A rise in blood ammonia leads to toxic levels of ammonia in the brain and contributes to the development of brain oedema [10]. Lactate is another pathogenic factor that was found to be elevated in association with a rise in ICP. The role of hyperlactataemia in the pathogenesis of HE remains elusive; however, it is believed to be ammonia dependent [11]. With the reproducible, consistent cause of death being intracranial hypertension, this large animal model of acetaminophen-induced ALF becomes an excellent paradigm to test many new treatments, including ammonia lowering strategies [12].

With the help of many research groups, the acetaminophen-induced ALF pig model has greatly evolved over the years. In a concerted effort, the critical care protocol has refined the cause of death to intracranial hypertension [7, 13]. Furthermore, not only is this an excellent model to study the early initiating stages of ALF but it is also a valuable model to investigate the complications of acetaminophen-induced ALF. However, a potential weakness in the proposed model would be its lack of reversibility, which warrants a thorough investigation. For example, it would be worth exploring whether attenuation of intracranial hypertension allows for liver regeneration/recovery to transpire. In conclusion, this reproducible large animal model of ALF is highly clinically relevant, and presents a broad therapeutic window for many of the associated complications, including intracranial hypertension.

REFERENCES
