Key Points in Establishing a Model of Mouse Liver Transplantation

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ABSTRACT

The explosion of interest in research into the mouse genome and immune system has meant that the mouse orthotopic liver transplantation (MOLT) model has become a popular means of studying transplantation immunity, organ preservation, ischemia-reperfusion injury, and surgical techniques, among others. Although numerous modifications and refinements of surgical techniques have simplified the operation, the relatively short duration of postoperative survival after MOLT remains an obstacle to longer-term follow-up studies. Here, we summarize the scientific basis of MOLT and our experience improving and refining the model in six key areas: anesthesia, operative technique, perfusion and preservation of the liver, cuff technique, anhepatic time, and the value of rearterialization for the liver graft. We also compare the characteristics of different surgical techniques, and give recommendations for the best means of tailoring technique to the objectives of a study. In doing so, we aim to assist other investigators in establishing and perfecting the MOLT model in their routine research practice.

THE RAT orthotopic liver transplantation (OLT) model, first reported by Lee et al in 1973, is practical and useful for the study of liver transplantation, but has several limitations [1]. Advances in microsurgery and development of the cuff technique led to the first description of mouse OLT by Qian18 years later [2,3]. The availability of genetically modified strains and knockout mice means that mouse OLT has become an increasingly attractive research tool for the study of liver transplantation immunology, hepatic ischemia-reperfusion injury (IRI), post-transplantation tumor recurrence or hepatitis virus infection, and many others [4–7]. Although there are some similarities with rat OLT, the smaller size and the lack of tolerance of the anhepatic phase mean that mouse OLT is a substantially more complicated and technically demanding procedure [3]. Only a handful of transplantation research centers are capable of establishing the mouse OLT model reliably and reproducibly. In this article, we examine and summarize the scientific literature on mouse OLT and our experience of approximately 1,000 procedures in six aspects.

ANESTHESIA

The liver is the principal organ for metabolizing and inactivating drugs and preparing them for excretion. It is susceptible to injury induced by anesthetics that undergo hepatic metabolism [8]. Furthermore, the anesthetic dose has to be altered depending on mouse strain, sex, age, weight, mutation, duration of surgery, and depth of anesthesia [9]. Administering anesthesia for mouse OLT without impairing the animal's metabolic and physiologic homeostatic mechanisms and consequently achieving optimal surgical outcomes is a considerable challenge.

Inhalational anesthesia with a mixture of isoflurane and oxygen provided by an automatic delivery system is now widely used for general anesthesia in mouse OLT [10–12], although methoxyflurane and ether have also been used [3,13]. An intraperitoneal injection technique using a balanced combination of ketamine–xylazine–acepromazine has also been described, with no apparent adverse effect on graft function, hepatic regenerative capacity, or 7-day survival after mouse 50% small-for-size liver transplantation [14].

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Nonetheless, isoflurane has become the standard inhalational anesthetic in current mouse OLT research. It has many advantages: it has low hepatotoxicity, is excreted rapidly, is easy to regulate depth of anesthesia, and it is possible to prolong anesthesia if necessary without increasing risk or causing cardiopulmonary depression, resulting in improved safety and shorter recovery times [15]. The control and monitoring of isoflurane anesthesia can be particularly difficult and time-consuming for investigators, especially in the training period, and isoflurane has the disadvantage of causing peripheral vasodilatation, which is sometimes not tolerated if severe hypotension develops in the anhepatic phase [16,17]. The influence of isoflurane on hemodynamic, cardiopulmonary, and physiologic functions means that the target inhaled concentration should be 3% to 4% in the induction phase, 2% in the maintenance phase, and <0.5% in the anhepatic phase [14].

Anesthesia using injectable agents is cheap, straightforward, allows the animal's position to be adjusted easily during surgery, and does not require exhaled gas scavenging [18,19]. There are, however, many disadvantages to the injectable anesthetic technique in mouse OLT. A substantial proportion of injectable anesthetics undergo hepatic metabolism and can modify hepatic hemodynamics, alter carbohydrate metabolism, or significantly alter hepatic and cardiopulmonary functions [20]. Despite previous reports that injected ketamine-xylazine-acepromazine is a simpler alternative to inhalational anesthesia and that ketamine has hepatoprotective properties [14,21], all three drugs are metabolized in the liver and excreted in the urine, and consequently increase the metabolic burden on the hepatorenal system [18]. In our experience, the recovery time after intraperitoneal anesthesia is also relatively long, increasing the risk of early postoperative death. In our opinion, intraperitoneal injection anesthesia should be regarded as a suitable technique for investigators training in mouse OLT, allowing them to focus on developing surgical skills rather than being distracted by anesthesia. Furthermore, there is no need for scavenging to eliminate anesthetic vapors from the operating environment. Inhalational anesthesia is recommended for the late training phase because of its favorable recovery time and minimal effect on hepatic function.

OPERATIVE TECHNIQUE

Investigators must develop sufficient surgical skill to minimize trauma to the animal as much as possible; operating time, especially the duration of cold and warm liver ischemia, should be kept to a minimum. Secure anastomoses of the hepatic vessels and bile duct must be achieved to avoid hemorrhage, thrombosis, bile leakage, stenosis, necrosis, and distortion of the anatomy [3,22]. Hemodynamic and respiratory stability should be maintained by minimizing intraoperative blood loss, supplementing fluid losses in a timely fashion, and modulating the depth of anesthesia when appropriate.

It is not possible to avoid handling, compressing, and turning the liver while dissociating and transferring the graft. Such manipulations contribute to the formation of microthrombus and cause local anoxia. Release of vasoactive and pro-inflammatory factors by activated Kupffer cells further impair the hepatic microcirculation, establishing a vicious cycle of trauma to the hepatic parenchyma and vascular bed that ultimately results in IRI [23]. Manipulation of the liver should be avoided when possible; necessary manipulations should be made gently and skillfully with a wet swab, especially before liver lavage.

Every effort should be made to avoid heat loss and to maintain the animal's temperature as close to normal as possible by irrigating the abdominal cavity with warm fluid, infra-red light, electric blanket heating, reflective foil materials, and thermal gel packets [24,25]. Reductions in core temperature are associated with longer emergence from anesthesia and physiologic disturbances that increase the risk of cardiac arrhythmia, coagulopathy, and postoperative infection [26–28]. The conscious mouse relies on behavioral and autonomic responses to maintain a relatively constant body temperature that is lost during anesthesia and impaired during recovery. Peripheral vasoconstriction and thermogenesis are the primary autonomic responses to cold challenges [29,30]. Therefore, as well as providing a warm recovery environment, it is essential to ensure that the animal is supplied with sufficient energy to avoid postoperative death, especially in the first 24 hours after transplantation.

PERFUSION AND PRESERVATION OF THE LIVER

Liver IRI, immune rejection, organ perfusion, and preservation are the main contributors to graft damage and primary nonfunction after transplantation [31]. Remarkable progress has been achieved in the field since the first rat OLT was performed [32]. Research in experimental animals has been a major contributor to understanding how best to perfuse and preserve the graft, but despite these advances, there is unfortunately still no guarantee of optimal hepatic function after OLT [33]. As hepatic failure is so difficult to treat in the mouse, impaired liver function is directly associated with high morbidity and mortality.

The strategy used in mouse OLT to minimize ischemic injury is in vivo cold flushing through the portal vein (PV) and/or abdominal aorta [3,14,34], followed by cold static storage with chilled isotonic solutions, usually 0.9% NaCl solution [35,36], Ringer's solution [3,13], or University of Wisconsin (UW) solution [37,38]. To the best of our knowledge, there is no direct evidence to guide the choice of solution, so the choice tends to be based on the experience or preference of the investigator. We find that perfusion of the abdominal aorta can achieve more uniform, thorough, reliable, and controllable dual perfusion of the hepatic artery (HA) and PV than perfusion through the PV alone. Nevertheless, several studies have found that postoperative survival time after nonarterialized mouse OLT does not appear to be influenced by perfusion technique [3,34].