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A reliable scoring system after major liver resection in mice

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ABSTRACT

Background: Posthepatectomy liver failure and its transplant counterpart, small-for-size syndrome, remain significant limitations for liver resections and segmental liver transplantation. Partial hepatectomy in mice is one of the most commonly used models to study liver regeneration, but blood and tissue sampling necessary to collect data can affect outcomes or even require euthanasia. We therefore developed a quantitative observational system to predict death from hepatectomy during the first 24 postoperative hours.

Materials and methods: A total of 100 female, 10 to 12-week-old C57BL/6 mice underwent two-thirds hepatectomy and were monitored for up to 7 d. Our scoring system was based on five categories, each assigned 0-2 points: activity level, body posture, fur condition, respiratory status, and eye appearance. Seventy-five mice were scored 6 h, 12 h, 24 h, 2 d, 3 d, 5 d, and 7 d after surgery. The remaining 25 mice were scored similarly, but underwent, in addition, blood sampling for serum alanine aminotransferase, total bilirubin, interleukin-6, tumor necrosis factor-alpha, or euthanasia with liver sampling for conventional hematoxylin-eosin and Ki-67 staining.

Results: Retrospective analysis indicated that body condition scores $\leq$5 on two consecutive time points within the first 24 postoperative hours accurately predicted eventual death. Animals in the low scoring group also had significantly higher serum alanine aminotransferase, total bilirubin, interleukin-6, tumor necrosis factor-alpha, more hepatocyte necrosis in hematoxylin-eosin, and fewer Ki-67 positive hepatocytes.

Conclusions: Our scoring system accurately predicts survival, hepatocyte damage, liver regeneration, and systemic inflammation in a mouse hepatectomy model, within the first 24 hours of surgery. This could be useful in evaluating posthepatectomy interventions for their effect on survival and liver regeneration.

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Introduction

Liver resection remains the treatment of choice for most hepatobiliary malignancies.[1] Posthepatectomy liver failure is a feared complication and major cause of perioperative mortality.[2] An excessive inflammatory response and impairment of liver regeneration play important roles in the development of posthepatectomy liver failure.[3] Two-thirds partial hepatectomy in mouse is one of the most commonly used animal models to study posthepatectomy liver function and regeneration because of its simplicity and repeatability.[4,5] Important advantages of the mouse model over large animal models include the availability of various genetic modified strains for mechanistic study, and the ability to repeat experiments quickly, in a standardized fashion.[6]

Investigators have endeavored to simplify and standardize animal procedures and perioperative management.[7,8] Perioperative monitoring of animals is part of all study protocols, but to date, there is no standardized observational assessment of animals subjected to major surgery. Although invasive tests, such as blood serum markers, are useful, during the acute postoperative phase, the animals are in critical condition and the additional trauma of handling and collection of blood in volume large enough to test may impact the animals' subsequent serum biomarker levels or even their survival, rendering post-testing outcomes difficult to interpret.[9] At the same time, euthanasia of animals in a moribund state is necessary not only for the welfare of animal but also for prenecropsy blood and tissue sample collection. A standardized observational assessment of the animals that could substitute for invasive monitoring would therefore be helpful in the investigation of survival and liver regeneration after partial hepatectomy. In this study, we developed and validated a quantitative body condition scoring system for animals after major liver resection.

Materials and methods

Animals

A 10- to 12-week-old female C57BL6 mice weighing 19-23 g were purchased from Jackson Lab (Bar Harbor, ME). Animals were maintained in a temperature-controlled specific-pathogen-free facility on a 12 h light–dark cycle with free access to water and standard chow and daily monitoring by veterinarians from the Center of Comparative Medicine in Massachusetts General Hospital. Mice were acclimated for 2 wk before surgery. Any animal that became moribund (see following descriptions) during the study was euthanized through isoflurane anesthesia and cervical dislocation. All animals that survived to postoperative day eight were euthanized in the same manner. All experiments were approved by the Institutional Animal Experiment Committee and in accordance with the Guide for the Care and Use of Laboratory Animals (eighth Ed., Washington, 2011).

Procedure and perioperative management

All procedures were carried out between eight and 11 in the morning without fasting before surgery. Livers of 10 mice from the same strain were resected to confirm the weight percentage of each lobe. The left lateral lobe (LLL) and median lobe (ML) constitute 65.3% of the liver (LLL: 32.3% ± 4.2%; ML: 33.1% ± 2.8%; right upper lobe: 16.0% ± 2.4%; right lower lobe: 11.6% ± 2.5%; anterior caudate lobe: 2.7% ± 0.8%; and posterior caudate lobe: 4.4% ± 1.4%). In a classic murine major liver resection model, two-thirds of the liver, the LLL and ML, were removed using the ligation technique.[7] Because of the hepatotoxicity of the most intraperitoneal or intravenous anesthetics, we used isoflurane inhalation (3.0% for induction and 2.0% for maintenance) with an oxygen flow rate of 4 L/min. A warming pad was placed under the mouse to prevent hypothermia, and a sterile 3 mL syringe was placed under the scapula to project the liver anteriorly for a better exposure. After shaving, betadine wiping and sterilely draping of the abdomen, a 1.5-cm upper midline incision was made, and an Alm retractor (Roboz, Germany) was used to expose the liver. The perihepatic ligaments were sharply divided. Using moistened Q-tips and forceps, the LLL was lifted and encircled with a 5-0 silk suture. The suture was tied at the base of the lobe. The lobe was excised distal to the suture using microdissecting scissors. The same was performed with ML. We routinely ligated the common base of the two parts of the ML (named as the left and right upper lobes and ligated separately in some literature[7]) simultaneously, taking care not to place the tie too close to the paracaval portion of ML, thus avoiding caval stricture. On closure of the abdomen, mice were given 0.5 mL of sterile saline containing 10% dextrose intraperitoneally to replenish fluid losses and prevent hyperglycemia. Animals were monitored using our cage-side observational scoring system at postoperative 6 h, 12 h, 24 h, and daily thereafter. A second dose of saline with 10% dextrose was given at 12 h routinely, and a third at 24 h, when necessary. Buprenorphine (0.1 mg/kg) was given subcutaneously immediately after operation, at 12 h and 24 h for analgesia.

Body condition scoring system

Cage-side observations by researchers were made in parallel with the veterinarians from Center of Comparative Medicine. The mice were scored at postoperative 6 h, 12 h, 24 h, 2 d, 3 d, 5 d, and 7 d. The current scoring system, we developed for the hepatectomized murine model was modified from those developed for tumor or total body irradiation studies.[10,11] Cages were removed from racks to facilitate better visualization of animals. The mouse was gently handled at the base of tail when necessary to visualize the abdominal fur. Mice received a score of 0 to 2 for each of the following categories: activity level, body posture, fur condition, respiratory status, and eye appearance (Table).

Activity level A score of 0 indicated an animal that was reluctant to move cowered in the corner of the cage and unresponsive to touch. One indicated an animal that was reluctant to move spontaneously, but moved away from contact, often with impaired gait. Two indicated an animal that moved around the cage normally and actively.

Body posture A score of 0 indicated a severely hunched posture. One indicated a moderately hunched posture (status between zero and two). Two indicated a normally stretched posture (Fig. 1A and B).

Fur condition A score of 0 indicated that the fur on abdomen and butt were wet and unkempt. One indicated moderately