Liver Failure After Extended Hepatectomy in Mice Is Mediated by a p21-Dependent Barrier to Liver Regeneration

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BACKGROUND & AIMS: Extended liver resection leads to hepatic failure because of a small remnant liver volume. Excessive parenchymal damage has been proposed as the principal cause of this failure, but little is known about the contribution of a primary deficiency in liver regeneration. We developed a mouse model to assess the regenerative capacity of a critically small liver remnant. **METHODS:** Extended (86%) hepatectomy (eHx) was modified to minimize collateral damage; effects were compared with those of standard (68%) partial hepatectomy (pHx) in mice. Markers of liver integrity and survival were evaluated after resection. Liver regeneration was assessed by weight gain, proliferative activity (analyses of Ki67, proliferating cell nuclear antigen, phosphorylated histone 3, mitosis, and ploidy), and regeneration-associated molecules. Knockout mice were used to study the role of p21. **RESULTS:** Compared with pHx, survival of mice was reduced after eHx, and associated with cholestasis and impaired liver function. However, no significant differences in hepatocyte death, sinusoidal injury, oxidative stress, or energy depletion were observed between mice after eHx or pHx. No defect in the initiation of hepatocyte proliferation was apparent. However, restoration of liver mass was delayed after eHx and associated with inadequate induction of Foxm1b and a p21-dependent delay in cell-cycle progression. In $p21^{-/-}$ mice, the cell cycle was restored, the gain in liver weight was accelerated, and survival improved after eHx. **CONCLUSIONS: Significant parenchymal injury is not required for liver failure to develop after extended hepatectomy. Rather, liver dysfunction after eHx results from a transient, p21-dependent block before hepatocyte division. Therefore, a deficiency in cell-cycle progression causes liver failure after extended hepatectomy and can be overcome by inhibition of p21.**

Keywords: Liver Regeneration; Small-For-Size Syndrome; Extended Hepatectomy; Liver Regeneration Failure.

The liver has a unique capacity to regenerate after major tissue loss. However, a threshold exists for the amount of residual tissue below which the liver does not recover sufficiently to maintain vital function. Thus, after extensive liver resection, a transient or sometimes fatal form of hepatic failure may develop, characterized by prolonged hyperbilirubinemia, reduced liver function, and sometimes death within a few days. This entity, known as Small-For-Size Syndrome (SFSS), currently is untreatable and considerably limits surgical therapy of many liver diseases.[1,2]

The pathophysiology underlying SFSS remains largely unknown.[3] It is particularly unclear whether the impaired liver function is a result of disproportional tissue damage after resection or is caused by a primary failure in regeneration of the small remnant. To study liver failure after extended hepatectomy, several murine models have been developed and suggest the accruement of damage is the critical event leading to liver dysfunction after extensive tissue loss.[4–7] Accordingly, the presence of excessive postoperative parenchymal injury and a pronounced early mortality are features shared by all existing mouse models of extended hepatectomy. However, the hepatic injury observed in mouse models is of unproven physiological significance and may confound the intrinsic capacity of a small remnant to regenerate. To better investigate failure of liver regeneration as a mechanistic factor after extended hepatectomy, a mouse model is needed that is characterized by minimal tissue injury. Such a model could reduce selection bias owing to early mortality, and may reveal whether the small size per se imposes a barrier for liver to initiate and progress through the regenerative process. To this end, we designed a novel mouse model of liver failure after extended hepatectomy with the following aims: (1) to minimize collateral damage while maintaining features reminiscent of human SFSS, and (2) to investigate primary liver regeneration in a small liver remnant.

BASIC AND TRANSLATIONAL LIVER

Materials and Methods

Animals

All animal experiments were performed in accordance with Swiss Federal Animal Regulations and approved by the

Abbreviations used in this paper: ATP, adenosine triphosphate; eHx, extended 86% hepatectomy; LW/BW, liver weight to body weight ratio; mRNA, messenger RNA; PCNA, proliferating cell nuclear antigen; pH3, phosphohistone 3; pHx, partial 68% hepatectomy; SFSS, Small-For-Size Syndrome; TUNEL, terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling.

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Veterinary Office of Zurich. Animals aged 10–12 weeks were kept on a 12-hour day/night cycle with free access to food and water. C57Bl/6 mice were obtained from Harlan (Horst, The Netherlands); p21 knockout animals and corresponding wild types (B6129SF2/J) were obtained from Jackson Laboratories (Bar Harbor, ME).

Animal Surgery

Anesthesia was induced by isoflurane inhalation (2%–4%), and preoperative subcutaneous application of buprenorphine (0.1 mg/kg bodyweight). Anesthetic depth was monitored by clinical parameters (respiratory rate and depth, color of mucous membranes and inner organs, movement, and reflexes). After surgery, animals were allowed to recover on a warming pad in a separate cage until completely conscious. For standard hepatectomy (partial 68% hepatectomy [pHx]), a midline incision was performed, and the liver was freed from ligaments. The pedicle of the left lobe was ligated (silk, 6/0), and resected. After cholecystectomy (Prolene, 8/0; Ethicon, Neuchatel, Switzerland), the middle lobe was ligated in 2 steps (silk 6/0) and resected. For extended hepatectomy (extended 86% hepatectomy [eHx]), all segmental portal vessels of the caudate, right anterior, left, and middle lobes were ligated individually (Prolene 8/0) (Figure 1*A* and *B*). The parenchyma was transected with silk 6/0 ligatures afterward. Vascular and biliary structures of the right posterior lobe were preserved by this technique and visually controlled.

Liver Weight to Body Weight Ratio

Liver regeneration was expressed by the ratio of liver weight to body weight (LW/BW). Animal weight was measured directly before harvesting.

Histologic Examination

H&E was performed on 3-μm, paraffin-embedded sections of the liver. DNA fragmentation was visualized with fluorescein–deoxyuridine triphosphate (terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling [TUNEL]) using a Cell Death Detection Kit (Roche Applied Science, Basel, Switzerland). Spleen sections served as TUNEL-positive controls. Immunostainings were performed for Ki67 (Abcam, Cambridge, United Kingdom), phosphohistone 3 (pH3; Chemicon, Darmstadt, Germany), Mpo (NeoMarkers, Fremont, CA), F4/80 (BMA Biomedicals, Augst, Switzerland), Vegfr3, Cd31 (BD Pharmingen, Allschwil, Basel), p27 (Santa Cruz, Santa Cruz, CA), and proliferating cell nuclear antigen (PCNA; Abcam) using the Ventana Discovery automated staining system and the iView DAB kit (Ventana Medical Systems, Tucson, AZ), and counterstained with hematoxylin. The number of Ki67-positive and pH3-positive hepatocytes was determined by manual counting in 20 random visual fields (200×). To better visualize hepatic sinusoids (Vegfr3, Cd31), images were pseudocolored by desaturating and brightening all color unrelated to brown (DAB), followed by color inversion using Adobe Photoshop (Adobe, San

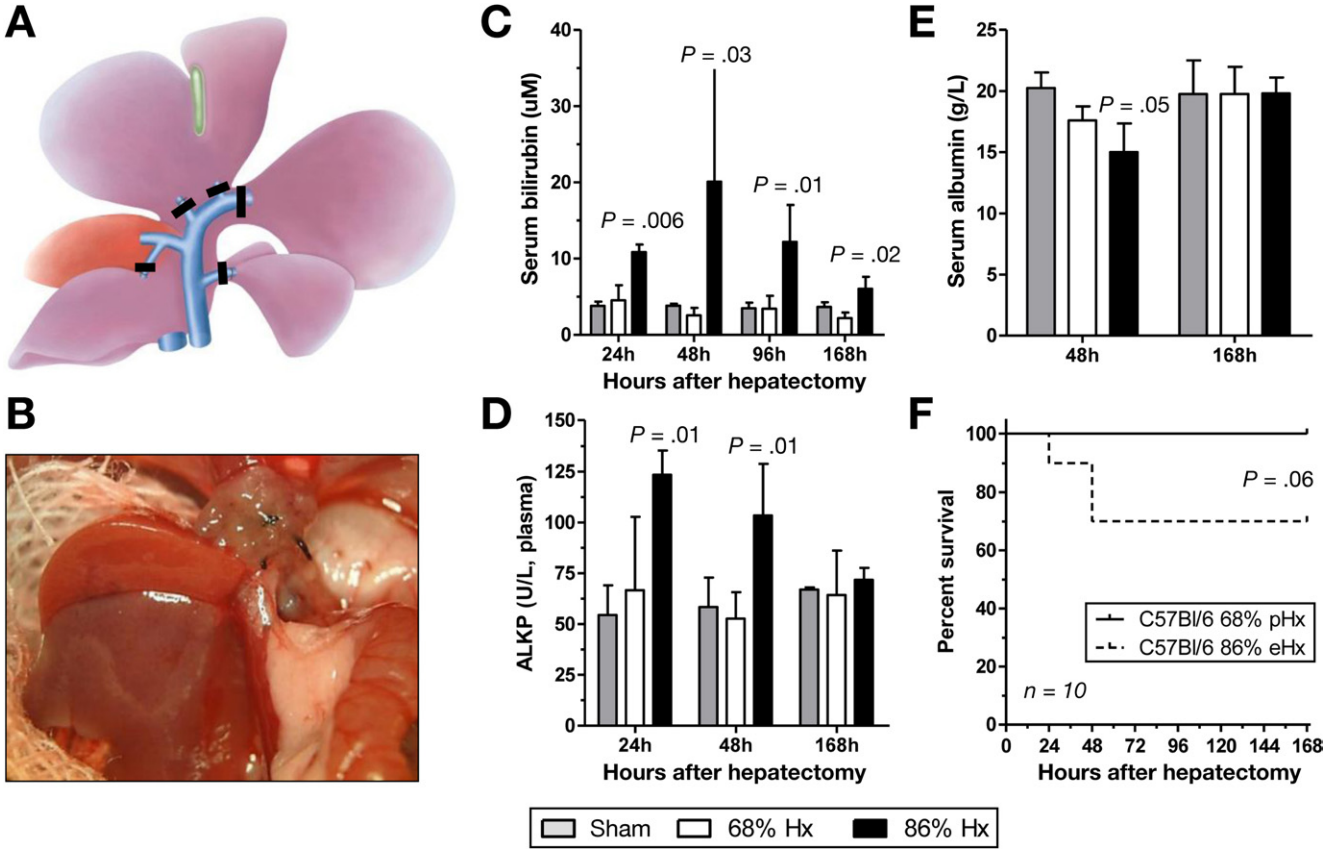

Figure 1. Extended hepatectomy induces features of liver failure in mice. (*A*) Modified surgical procedure for the radical resection of mouse liver. Segmental portal vein branches are ligated separately before parenchymal transection. (*B*) Ischemia of the ligated anterior right liver lobe before parenchymal resection. (*C*) Serum bilirubin levels at 24 to 168 hours after resection. (*D*) Plasma alkaline phosphatase (ALKP) levels at 24, 48, and 168 hours after resection. Note the similar patterns for bilirubin and ALKP levels. (*E*) Serum albumin levels at 48 and 168 hours after resection. (*F*) Kaplan–Meier estimates for the survival of mice after standard (pHx) or the modified extended (eHx) resection.