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Postponing the Hypoglycemic Response to Partial Hepatectomy Delays Mouse Liver Regeneration

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All serious liver injuries alter metabolism and initiate hepatic regeneration. Recent studies using partial hepatectomy (PH) and other experimental models of liver regeneration implicate the metabolic response to hepatic insufficiency as an important source of signals that promote regeneration. Based on these considerations, the analyses reported here were undertaken to assess the impact of interrupting the hypoglycemic response to PH on liver regeneration in mice. A regimen of parenteral dextrose infusion that delays PH-induced hypoglycemia for 14 hours after surgery was identified, and the hepatic regenerative response to PH was compared between dextrose-treated and control mice. The results showed that regenerative recovery of the liver was postponed in dextrose-infused mice (versus vehicle control) by an interval of time comparable to the delay in onset of PH-induced hypoglycemia. The regulation of specific liver regeneration–promoting signals, including hepatic induction of cyclin D1 and S-phase kinase-associated protein 2 expression and suppression of peroxisome proliferator-activated receptor γ and p27 expression, was also disrupted by dextrose infusion. These data support the hypothesis that alterations in metabolism that occur in response to hepatic insufficiency promote liver regeneration, and they define specific pro- and antiregenerative molecular targets whose regenerative regulation is postponed when PH-induced hypoglycemia is delayed. *(Am J Pathol 2016, 186: 587–599; http://dx.doi.org/10.1016/j.ajpath.2015.10.027)*

Recovery from any serious injury to the liver depends on the intrinsic ability of this organ to regenerate.[1] Thus, the signals that control liver regeneration have been subjected to extensive investigation with the hope that new mechanistic knowledge can be translated into improved clinical management of liver diseases in humans. Mouse two-thirds partial hepatectomy (PH) is the experimental paradigm most commonly used for studying liver regeneration. Analyses using PH have shown that partial liver resection induces a characteristic hepatocellular proliferative response that is precisely controlled by specific cytokines, growth and transcription factors, and intracellular signaling events.[1] This process ultimately restores normal hepatic mass and function, after which hepatocytes return to their pre-regenerative state of proliferative inactivity.[1] Nonetheless, the earliest events that initiate hepatic regeneration and later signals that terminate this process remain incompletely defined, and translation of mechanism-based, proregenerative interventions into new treatments for liver diseases has not yet been achieved.

In addition to initiating hepatic regeneration, severe liver injuries alter metabolism.[2–4] For example, mice subjected to PH develop hypoglycemia, followed by systemic catabolism and humoral and hepatic accumulation of specific metabolites.[5–8] These stereotypical changes are detectable almost

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immediately after surgery and resolve with regenerative recovery of the normal liver/body mass ratio. Moreover, some experimental manipulations that suppress PH-induced alterations in metabolism impair liver regeneration,[5–7] and other interventions that amplify elements of this metabolic response accelerate regeneration.[3] Together, those observations implicate the metabolic response to hepatic insufficiency as an important source of proregenerative signals. However, the specific molecular mechanisms by which metabolism controls liver regeneration remain poorly understood. The studies reported here were undertaken to clarify the functional importance and mechanistic role of PH-induced changes in glycemia during normal mouse liver regeneration, using parenteral dextrose supplementation to delay the onset of such hypoglycemia.

Materials and Methods

Partial Hepatectomy

PH was performed on 2- to 3-month-old male C57BL6/J mice (Jackson Laboratory, Bar Harbor, ME). Animals were maintained on 12-hour dark/light cycles with *ad libitum* access to standard rodent chow and water before and after surgery. At the time of surgery, mice were sedated with inhaled isoflurane (Vedco Inc., St. Joseph, MO) via an anesthesia vaporizer and then subjected to midventral laparotomy with exposure, ligation, and resection of the left and median hepatic lobes, followed by closure of the peritoneal and skin wounds as described previously.[5–16]

Parenteral Dextrose Infusion

At the time of PH, one end of a sterilized catheter prefilled with sterile 22.5% dextrose [45% sterile dextrose (Sigma-Aldrich, St. Louis, MO) diluted 1:1 with phosphate-buffered saline] or half-strength phosphate-buffered saline (diluted with sterile water instead of dextrose) as control was implanted into the peritoneal cavity and the other end tunneled s.c. through the skin in the back of the neck and attached to a peristaltic pump (Harvard Apparatus, Holliston, MA). Pilot studies were initially conducted to compare PH-induced changes in blood glucose between mice treated with glucose infusion rates (GIRs) from 20 to 35 μg of glucose per gram of body weight (μg/g) per minute and controls administered an equal volume of vehicle. Those studies showed that provision of a GIR of 25 μg/g per minute (160 mL/kg per day) beginning immediately after PH, and subsequent adjustment of the GIR every 2 hours (based on glucometric analyses of tail vein blood) up or down by 5 μg/g per minute between 20 and 35 μg/g per minute (in an effort to maintain blood glucose within the normal, strain-specific, nonfasting range of 198 ± 14 mg/dL; Mouse Phenome Database, *http://phenome.jax.org/db/qp?rtn=views/measplot&brieflook=32301*, last accessed October 19, 2015, MPD:32301) reproducibly delays the onset of PH-induced hypoglycemia for 14 hours after surgery. This regimen of parenteral dextrose supplementation and glucose monitoring was used in all subsequent analyses. No postoperative mortality was observed in 53 control mice; however, 2 of 75 dextrose-infused animals died (one on the day of surgery and the other on postoperative day 1). There were no differences in postoperative morbidity (eg, decreased activity, jaundice, extreme weight loss, or piloerection) between the dextrose-infused and control mice. Therefore, such morbidity (which affected a minority of animals) was interpreted as a technical complication of the model, and data from morbid animals were excluded from subsequent analyses. All other animals remained well-appearing throughout the experiment and were sacrificed at serial times after surgery for serum or plasma and tissue harvest as described previously.[1,5–16] Blood glucose profiles were generated with the glucometric data obtained from all nonmorbid animals. For other analyses, serum, plasma, or tissue samples from three to eight animals at each time point and in each treatment group were studied.

All experiments were approved by the Washington University Animal Studies Committee, and all animals received humane care in accordance with institutional guidelines and the criteria outlined in the Guide for Care and Use of Laboratory Animals (prepared by the National Academy of Sciences and published by the NIH; publication 86-23, revised 1985).

Histological Examination and IHC Analysis

Liver histological examination, hepatocellular bromodeoxyuridine (BrdU) incorporation, and immunohistochemistry analysis for cyclin D1 were assessed as described previously.[1,5–18] For BrdU analyses, animals were administered an i.p. injection of 100 mg/kg of BrdU (Sigma-Aldrich) 1 hour before sacrifice. Formalin-fixed, paraffin-embedded liver tissue was stained either with hematoxylin and eosin or for nuclear BrdU incorporation. Hepatocellular nuclear BrdU labeling and mitoses were quantified by examination of at least three random ×400 fields and at least 300 cells and nuclei in each tissue section. Primary antibodies for immunohistochemistry analysis included BrdU (catalog number OBT0030; Accurate Chemical & Scientific Corp., Westbury, NY) and cyclin D1 (catalog number RB-9041; Thermo Scientific, Rochester, NY).

Serum Metabolite and Plasma Hormone Analyses

Concentrations of serum amino acids were determined by the St. Louis Children's Hospital Core Laboratory as previously described.[19–21] Serum free fatty acids and hepatic triglycerides were quantified as previously described.[10] Plasma insulin was determined by quantitative fluorescent immunoassay (Singulex, Alameda, CA) and glucagon by radioimmunoassay (Millipore, Billerica, MA) according to the manufacturer's instructions for each assay, by the