A new technique for accelerated liver regeneration: An experimental study in rats

Kasper Jarlhelt Andersen, MD, PhD,[a] Anders Riegels Knudsen, MD, PhD,[a] Betina Norman Jepsen, MD,[a] Michelle Meier, MD,[a] Anders Patrik Alexander Gunnarsson, MSc,[b,c] Uffe Birk Jensen, MD, PhD,[b,c] Jens Randel Nyengaard, MD, DMSc,[d] Stephen Hamilton-Dutoit, MD, FRCPath,[e] *and* **Frank Viborg Mortensen, MD, DMSc,[a]** *Aarhus, Denmark*

Background. *Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) is used to accelerate growth of the future liver remnant. We investigated alternative methods for increasing the future liver remnant.*

Methods. *A total of 152 rats were randomized as follows: (1) sham; (2) portal vein ligation; (3) portal vein ligation/surgical split (ALPPS); (4) portal vein ligation/split of the liver with a radiofrequency ablation needle; (5) portal vein ligation/radiofrequency ablation of the deportalized liver (portal vein ligation/radiofrequency ablation necrosis in the deportalized liver); (6) portal vein ligation/radiofrequency ablation of the future liver remnant (portal vein ligation/radiofrequency ablation–future liver remnant); and (7) controls. Animals were evaluated on postoperative days 2 and 4. Bodyweight, liver parameters, hepatic regeneration rate, proinflammatory cytokines, hepatocyte proliferation, and gene expression were measured.*

Results. *Hepatic regeneration rate indicated a steady increase in all intervention groups compared with sham rats (*$P < .001$*). At postoperative day 2, the hepatic regeneration rate was significantly higher in the portal vein ligation/radiofrequency ablation necrosis in the deportalized liver group than in the portal vein ligation group (*$P = .039$*). On postoperative day 4, we found significant differences between the portal vein ligation group and the ALPPS (*$P = .015$*), portal vein ligation/split of the liver with a radiofrequency ablation needle (*$P = .010$*), and portal vein ligation/radiofrequency ablation necrosis in the deportalized liver (*$P = .046$*) groups. Hepatocyte proliferation was significantly higher at all times compared with sham rats. On postoperative day 4, we found a significantly higher proliferation in groups 3, 4, 5, and 6 compared to portal vein ligation. Gene analysis revealed upregulation of genes involved in cellular proliferation and downregulation of genes involved in cellular homeostasis in all intervention groups. Between the intervention groups, gene expression was nearly identical. Biochemical markers and proinflammatory cytokines were comparable between groups.*

Conclusion. *The surplus liver regeneration after ALPPS is probably mediated through parenchymal damage and subsequent release of growth stimulators, which again upregulates genes involved in cellular regeneration and downregulates genes involved in cellular homeostasis. We also demonstrate that growth of the future liver remnant, comparable to that seen after ALPPS, could be achieved by radiofrequency ablation treatment of the deportalized liver, that is, a procedure in which the initial step in humans can be performed percutaneously. (Surgery 2017;■:■-■.)*

From the Department of Surgery,[a] Section for Upper Gastrointestinal and Hepato-Pancreato-Biliary Surgery, and the Department of Clinical Genetics,[b] Aarhus University Hospital; the Institute of Clinical Medicine,[c] Aarhus University; and the Stereology & Electron Microscopy Laboratory,[d] Centre for Stochastic Geometry and Advanced Bioimaging, and the Institute of Pathology,[e] Aarhus University Hospital, Aarhus, Denmark

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Reprint requests: Kasper Jarlhelt Andersen, MD, PhD, NVL Research, Nørrebrogade 44, Aarhus C, 8000 Denmark. E-mail: kasperjarlheltandersen@gmail.com.

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Liver resection remains the standard for treatment of hepatic malignancies. Liver resection can be performed if the volume of the future liver remnant (FLR) is 25% of the total initial volume or greater. Unfortunately, many cancer patients have multiple liver lesions at diagnosis. This makes radical resection impossible, because of a marginal FLR, increasing the risk of posthepatectomy liver failure. In recent years, several techniques have

been introduced, designed to increase the size of the FLR. These techniques include portal vein embolization (PVE),[1,2] portal vein ligation (PVL),[3] 2-stage hepatectomy,[4] and, recently, the associating liver partition with PVL for staged hepatectomy (ALPPS).[5]

Several technical variations of ALPPS have been described in the literature. Many of these techniques were reviewed in a recent paper by Edmondson et al,[6] including radiofrequency ablation (RFA)–assisted liver partition. The ALPPS procedure seems to be superior to the other techniques for achieving increased size and accelerated growth of FLR.[7] The major drawback of ALPPS, however, is that patients are subjected to 2 open operative procedures that are associated with high morbidity and mortality.[7]

Since the introduction of the ALPPS procedure in 2012 by Schnitzbauer and colleagues,[8] a few studies have investigated the mechanisms behind the accelerated liver regeneration. It has been suggested that the surplus growth is due to the cessation of collateral portal circulation between the normally perfused and deportalized parts.[9] Other studies and case-reports, however, indicate that the accelerated regeneration can be induced without compromising the collateral portal blood flow.[10-12] In these studies, the accelerated growth seems to be caused by an excessive systemic inflammatory response that is a consequence of the parenchymal damage induced by liver partition.[12] Whether the different methods of inducing accelerated regeneration have similar driving mechanisms and molecular pathways remains to be examined.

The main purpose of the present study was to investigate whether the accelerated growth in FLR after ALPPS can be achieved by other techniques that do not compromise the collateral blood flow between the deportalized and normally perfused parts of the liver. The modified procedures in the present study were designed so that the initial stage could be performed percutaneously in humans. This would presumably be of great benefit to patients in the clinical setting, as it could be expected to decrease the high morbidity and mortality associated with ALPPS. In addition, it was our purpose to investigate the inflammatory and genomic responses in our experimental animals in order to identify and compare potential key regulators of the additional accelerated liver growth.

EXPERIMENTAL PROCEDURES

Animals and ethics. The Danish Animal Experiment Inspectorate in Copenhagen, Denmark, approved this animal study under license no. 2015-15-0201-00580. The experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health. Male Wistar rats were obtained from Taconic (Borup, Denmark) and acclimated to the animal facility for one week prior to experimentation. All animals were housed in standard animal laboratories maintained at a temperature of 23°C with an artificial 12-hour, light-dark cycle. The animals had unlimited access to food (Altromin) and water. All rats were monitored daily for changes in weight, behavior, and physical appearance.

Experimental design. We used 152, 10-week-old male Wistar rats weighing 200 g. The animals were randomly assigned to the following groups:

1) Sham: Midline incision with no further treatment
2) PVL: Portal vein ligation
3) ALPPS: Portal vein ligation and surgical split of the liver
4) PVL/RFA-SPLIT: Portal vein ligation and split of the liver with an RFA needle
5) PVL/RFA-DEPOR: Portal vein ligation and RFA necrosis in the deportalized liver
6) PVL/RFA-FLR: Portal vein ligation and RFA necrosis in the future liver remnant
7) Control group: Healthy rats

The groups and each procedure will be described further in the following. Afterward, the animals were further randomly evaluated on either postoperative day (POD) 2 or POD 4. The choice of these PODs was based on a previous study, which showed days 2 and 4 to be key points in rat liver regeneration.[13] Blood and liver tissue were sampled before the rats were killed.

Anesthetics and analgesia. Animals were anesthetized in a glass cylinder filled with a mixture of oxygen (2.0 l/minute), N_2O (0.5 l/minute), and 4% sevoflurane (Forene, Abbott Laboratories, UK). During surgery, anesthesia was maintained with 2% sevoflurane, oxygen, and N_2O administered through a nasal mask.

Surgical procedure. The animals were placed in a supine position on a heated pad and injected with the long-lasting non-steroidal anti-inflammatory drug, Carprofen (Rimadyl; Pfizer Animal Health, Exton), at a dose of 5 mg/kg in 1.0 mL isotonic saline. Animals were then given an injection with isotonic saline at a dose of 1 mL/100 g body weight. Finally, a midline abdominal incision was made and the liver mobilized (sham). All ligaments were then cut to avoid collateral blood flow.