Effect of different liver resection methods on liver damage and regeneration factors VEGF and FGF-2 in mice

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Background: Different approaches to study liver regeneration in murine models have been proposed. We investigated the effect of different liver resection models on liver damage and regeneration parameters in mice.

Methods: We compared the technical aspect of the 2 most commonly used techniques of 50% and 70% liver resection. Liver damage, as determined by the change in serum alanine aminotransferase and aspartate aminotransferase, as well as the regeneration parameters VEGF and FGF-2 were analyzed at 6 time points. A postoperative vitality score was introduced.

Results: Cholestasis was not observed for either technique. Both resection techniques resulted in full weight recovery of the liver after 240 hours, with no significant difference between sham and resection groups. Postoperative animal morbidity and total protein levels did not differ significantly for either method, indicating early and full functional recovery. However, comparing the mitogenic growth factors FGF-2 and VEGF, a significant increase in serum levels and, therefore, increased growth stimulus, was shown in the extended resection group.

Conclusion: Extended resection led to a greater response in growth factor expression. This finding is important since it shows that growth factor response differs acdording to the extent of resection. We have demonstrated the need to standardize murine hepatic resection models to adequately compare the resulting liver damage.

Contexte : Différentes approches ont été proposées pour étudier la régénérescence du foie dans des modèles murins. Nous avons exploré l'effet de divers modèles de résection sur l'atteinte hépatique et les paramètres de la régénérescence chez la souris.

Méthodes : Nous avons comparé l'aspect technique des 2 approches les plus couramment utilisées, soit les résections de 50 % et de 70 % du foie. L'atteinte hépatique, déterminée en fonction de la modification des taux d'alanine aminotransférase et d'aspartate aminotransférase sériques, ainsi que les paramètres de régénérescence VEGF (facteur de croissance endothéliale vasculaire) et FGF-2 (facteur de croissance des fibroblastes) ont été analysés à 6 moments. Un score de vitalité postopératoire a en outre été introduit.

Résultats : Aucune des 2 techniques de résection n'a donné lieu à la cholestase. Les 2 techniques ont produit un rétablissement entier du poids des foies après 240 heures, sans différence significative entre les groupes soumis à l'intervention factice ou réelle. La morbidité et les taux de protéines totales postopératoires ont été relativement semblables avec les 2 techniques, attestant d'un rétablissement fonctionnel rapide et complet. Toutefois, en comparant les facteurs de croissance mitogènes FGF-2 et VEGF, nous avons observé une augmentation significative des taux sériques; nous en avons conclu que le stimulus de croissance était plus marqué dans le groupe soumis à la résection étendue.

Conclusion : La résection étendue a donné lieu à une expression plus marquée des facteurs de croissance. Cette observation est importante car elle montre que la réponse des facteurs de croissance diffère selon l'étendue de la résection. Nous avons démontré qu'il faut standardiser les modèles de résection hépatique murins pour pouvoir comparer adéquatement l'atteinte hépatique qui en découle.

Several rodent models for hepatic resection to investigate liver regeneration and repair after liver injury, as well as cell cycle dynamics, have so far been established.[1–4] The availability of multiple strains of genetically engineered mice has shifted the focus from the rat toward the murine model.[2]

In 1931, Higgins and Anderson[5] described the classic rodent model for hepatic regeneration in rats in which 2 of 4 liver lobes, about two-thirds of the rat liver, were removed. This approach, however, is not feasible in mice since the rat liver is composed of 4 lobes, whereas the murine liver contains 7 lobes.[6,7] Liver resection in mice is technically more demanding than resections in rats because of liver size, tissue texture and the lack of invasive monitoring.[6] Presently, extended hepatic resections in mice are generally not yet performed in a standardized fashion, and therefore such studies in mice are systematically biased.

The real extent of resection with removal of functioning parenchyma is of importance because of a different regeneration stimulus and potency of the remaining liver.[2,4]

Nevertheless, numerous groups[1,8–10] conducting murine studies still use modifications of the technique originally described by Higgins and Anderson for rats, meaning the resection of the 2 left lateral lobes of the liver, thus mistakenly implying the same amount of resected tissue as in rats.

We conducted the present study to investigate the effect of different liver resection models on liver damage and regeneration parameters in mice and help establish a safe, reproducible and extended hepatectomy (70% liver weight). This facilitates standardized regeneration analysis. Furthermore, Sato and colleagues[11] showed the importance of the mitogenic growth factors FGF-2 and VEGF, in hepatic regeneration.

Methods

Animals

We obtained 8- to 10-week-old male Balb-c mice weighing 20–25 g from Janvier, Le Genest St. Isle, France. All mice were bred with an alternating 12:12-hour light:dark cycle under conditions of controlled temperature and free access to standard food and water in the animal care facility of the Medical Research Center, University of Heidelberg.

Experiments were performed in accordance with German legislation on the protection of animals and the guide of care and use of laboratory animals (NIH publication 86–23, revised edition, 1985).

Surgery

Mice were anesthetized by intraperitoneal injection of 2.25 mg/g of xylazine (Rompun 0.1%, Bayer Vital GmbH) and 150 mg/g of ketamine (Hostaket S 0.25%, Hoechst Roundel Vets) in sodium chloride (0.9%) at a volume ratio of 1:1:3.

The mice underwent a sham operation, 50% hepatectomy (method 1) or 70% hepatectomy (method 2).

In a pilot group, we determined the relative weight of the 7 isolated lobes in relation to the whole liver. The single fractional contributions to the total liver weight of the single lobes did not differ substantially from previous results by other authors.[6,7]

For resection, the abdominal skin was shaved. Mice were placed on a heating plate (37°C) throughout the perioperative period. A 1.5–2 cm upper midline incision was made beginning from the xyphoid. After opening the peritoneum, we administered 1 mL of warm 0.9% sterile saline to prevent dehydration and replenish fluid loss. With moistened Q-tips, preparation was performed, and the liver was gently mobilized by dissecting the falciform ligament. After exposure of the liver, the lobes were lifted with Q-tips and resected with 5–0 sutures (Ethicon) around the appropriate lobes close to the inferior vena cava. The hepatic lobes were removed distal to the applied ligatures.

Method 1 included the resection of the left lower and upper lobes (50%). In method 2, the right upper lobe was also removed (70%).

Before closure, the abdominal cavity was irrigated with 1 mL of sterile warm saline to decrease contamination and prevent dehydration. Muscle and skin were closed in 2 layers with 5/0 Vicryl and 6/0 Prolene (Ethicon).

For sham operations, mice were anesthetized, the abdomen was opened and the liver mobilized by dissecting the falciform ligament, without resection.

After the procedure, mice remained on a heating plate at a temperature of 37°C while waking up from anesthesia. Mice were monitored postoperatively and vitality was assessed. The vitality score was determined 1 hour after the operation as follows:

- perioperative death (vitality score 0);
- breathing, vibrissae/whisker movement (1);
- head movement (2);
- body movement (3);
- full mobility (4); and
- full recovery, feeding and drinking (5).

For postoperative analgesia, we injected buprenorphine (0.1 mg/kg) subcutaneously into the neck fat pad.

Mice were sacrificed at 10 minutes, or 3, 24, 48, 120 or 240 hours after hepatectomy under general anesthesia by cervical dislocation. The liver weight was determined immediately after death.

Serum analysis

Collected blood was immediately placed on ice before being centrifuged at 3000 rpm for 10 minutes to extract the serum. Choice of growth factors and liver damage parameters was based on the literature and on the accessibility of analysis equipment.

We measured VEGF and FGF-2 protein levels using an enzyme-linked immunosorbent assay (ELISA) detecting all isoforms of the respective proteins in solution (R&D Systems). Assays were performed in duplicate using 200 µL per well. Protein was extracted by incubation of the homogenized samples in tris-buffered saline (pH 8.5)

containing 0.5% aproptinin and 1% Triton X (Sigma-Aldrich) for 12 hours at 41°C. Samples were centrifuged at 100 000*g* for 60 minutes, and we determined protein concentration using a bicinchoninic acid protein assay. Samples used for ELISA contained 30–40 mg/mL protein. Values were read on a microplate spectrophotometer (Diagnostic Products Corporation) at 450 nm. Using recombinant protein (included in the assay), values could be determined from the standard curve and the picogram per milligram of protein could be calculated. The limits of detection were determined to be 7.8–500 pg/mL.

Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin and total protein were measured using Hitachi Autoanalyzer 911 (Roche) with a photometric enzymatic test system.

Statistical analysis

The data were analyzed using the Student *t* test for unpaired samples. Statistical analyses were performed with SPSS software version 11. We report data as means with standard deviations (SD). We considered results to be significant at $p < 0.05$ ($n = 10$ per group, survivors only).

Results

Animals were randomly divided into 3 groups. Partial hepatectomy was performed in 120 animals (60 animals each underwent methods 1 and 2). We recorded vitality scores of 3 or greater and survival in 118 animals. Using method 1, we resected a mean of 49.79% (SD 2.13%) of liver tissue compared with a mean of 70.04% (SD 6.83%) using method 2 ($p < 0.001$). The mean liver weight was 1.23 (SD 0.11) g. Technical complications owing to a defective heating plate after surgery resulted in vitality scores of 1 or 2 in 2 mice.

A sham operation was performed in 60 animals, with 1 perioperative death from intraoperative pneumothorax during the preparation of the falciform ligament. Procedure durations ranged from 10 to 15 minutes. The resection was completed within minutes. There was no significant difference in the mean postoperative vitality scores of mice that underwent method 1, method 2 or the sham operation. Later in the postoperative period (> 3 h), mice recovered completely with no signs of distress or changes in behaviour and food intake.

Because only small amounts of blood could be extracted owing to limited amounts of total mouse blood, the measurable markers of liver function were bilirubin, total protein, AST, ALT and the growth factors VEGF and FGF-2.

As indicators for liver damage, AST and ALT were quantified. The mean (and SD) liver enzymes in mice that underwent method 1 (ALT: 923.96 [250.09] U/L, AST: 907.05 [283.50] U/L) were significantly lower 3 hours after surgery (AST: $p = 0.048$, ALT: $p = 0.013$) than those of mice that underwent method 2 (ALT: 1446.96 [435.66] U/L, AST: 1235.19 [318.93] U/L), with the lowest levels measured in the sham group (ALT: 170.73 [121.51] U/L, AST: 235.65 [85.22] U/L; $p < 0.001$). After 48 hours, we found significant differences between method 1 and sham mice (ALT 127.04 [54.90] U/L, AST: 136.56 [46.26] U/L v. ALT: 36.5 [9.78] U/L, AST: 65.83 [14.27] U/L) and between method 2 (ALT: 181.26 [89.64] U/L, AST: 158.84 [70.92] U/L) and sham mice. After 10 minutes, 120 hours and 240 hours, no significant differences were found among the groups.

Bilirubin was not increased in any of the mice, confirming the lack of surgery-related cholestasis.

We found no significant difference in the weights of the regenerated livers of method 1 and 2 mice 240 hours after resection, nor did we find significant differences between treatment versus sham mice. The weight of the regenerated livers returned to normal in all groups. Synthesis parameters, such as total protein concentration, were similar in the 3 groups over time.

However, at 48 hours, the level of VEGF was significantly increased after method 2 (mean 57.17 [SD 5.31] pg/mL) compared with method 1 resection (mean 29.88 [SD 9.03] pg/mL) and sham operation (mean 22.61 [SD 7.80] pg/mL). We found no significant differences at the other time points.

The concentration of serum FGF-2 increased significantly only for method 2 resection (mean 39.49 [SD 11.20] pg/mL) compared with the sham operation (mean 12.29 [SD 11.35] pg/mL) and method 1 resection (mean 9.32 [SD 1.42] pg/mL) after 120 hours. We found no difference between method 1 resection and sham operation at this or any other time point.

Discussion

In 1931, Higgins and Anderson[5] proposed the classic model to study hepatic regeneration in rats, where 2 of 4 liver lobes are removed. These comprise about two-thirds of the rat liver. This approach is not feasible in mice owing to significant differences in anatomy and liver texture.[6] Whereas rats haven been preferred to mice to examine cross-hepatic regeneration, the availability of multiple transgenic strains has shifted the focus back to murine models.[2] Therefore, standardized partial hepatectomy models for mice are needed. Several groups have recently provided surgical techniques to consistently remove two-thirds of liver tissue.[2,4,6] Nevertheless, we know of no report in mice that would elucidate the effect of different liver resection methods on murine liver damage and regeneration parameters. Our study compared 50% and 70% resection models in mice.

In rodents, there is an association between liver growth and body mass. Removal of up to 30% of the liver fails to cause a synchronized wave of hepatocyte proliferation after

operation, although the liver eventually regains its mass.[3,12] In resections with removal of 40%–70% of the liver, there is a linear association between the amount of tissue resected and the extent of hepatocyte proliferation,[13] but resection of more than 70% results in increased mortality.[14] It has been demonstrated that in mice a 30% hepatectomy elicits the priming reaction, but fails to induce cell-cycle progression.[15] We found no difference in recovery after 240 hours between 50% and 70% resection. Protein synthesis was also at comparable levels at this end point.

However, hepatocytes are damaged by resection.[16] During replication, the remaining damaged hepatocytes have to compensate for increased metabolic demand and for the requirement to sustain or increase the level of vital serum proteins in the context of an acute-phase response.[10] The balance between replication, metabolism and synthesis ensures the survival of the affected animal. We monitored liver damage by levels of ALT and AST. Other than during the first 3 hours postoperatively, we found no difference between 50% and 70% resection, indicating a comparable level of damage to the residual liver.

The activation of proliferation and angiogenesis by FGF-2 and VEGF is essential for liver regeneration and organ repair, both of which depend on the supply of blood to hepatocytes.[17–19] The initial wave of hepatic proliferation is followed by endothelial cell proliferation[11] and penetration of avascular hepatocellular islands leading to formation of new sinusoids.[20] Regenerative factor VEGF stimulates endothelial cell proliferation and migration. Hepatocellular production of VEGF peaks 48–72 hours after hepatectomy,[20] as mitogens for hepatocytes FGF-2 are overexpressed in the regenerating liver.[21,22] It has been reported that FGF-2 stimulates the regeneration of the extracellular matrix after liver injury and regulates proliferation and migration of hepatocytes in vitro.[23,24] In the regenerating liver, FGF-2 seems to be primarily produced in hepatic stellate cells acting on the sinusoids.[11] Moreover, FGF-2 transmits its signals via an autocrine or a paracrine mechanism involving its high-affinity transmembrane receptors.[25,26]

Conclusion

We found that both important regenerative factors VEGF and FGF-2 are expressed differently only in response to a sufficient stimulus: 70%, but not 50%, resection. Not unexpectedly, extended resection led to a greater response in growth factor expression. This finding is of importance since it shows that growth factor response differs according to the extent of resection. We conclude that in models employing the inhibition of a sufficient growth factor response during liver regeneration, the described method 2 (70% resection model) should be used.

Competing interests: None declared.

Contributors: R. Bönninghoff, K. Schwenke, M. Keese, M. Otto and J. Sturm designed the study. R. Bönninghoff, K. Schwenke, R. Magdeburg, M. Otto and T. Hassenberg acquired the data, which was analyzed by R. Bönninghoff, K. Schwenke, M. Keese, H. Bitter-Suermann, M. Otto, T. Hassenberg and S. Post. R. Bönninghoff, K. Schwenke and M. Keese wrote the article, which was reviewed by R. Bönninghoff, K. Schwenke, R. Magdeburg, H. Bitter-Suermann, M. Otto, T. Hassenberg, S. Post and J. Sturm. All authors approved its publication.

References

1. Yamauchi H, Uetsuka K, Okada T, et al. Impaired liver regeneration after partial hepatectomy in db/db mice. *Exp Toxicol Pathol* 2003; 54:281-6.
2. Boyce S, Harrison D. A detailed methodology of partial hepatectomy in the mouse. *Lab Anim (NY)* 2008;37:529-32.
3. Martins PN, Theruvath TP, Neuhaus P. Rodent models of partial hepatectomies. *Liver Int* 2008;28:3-11.
4. Mitchell C, Willenbring H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. *Nat Protoc* 2008;3:1167-70.
5. Higgins G, Anderson G. Experimental pathology of the liver restoration of the liver of the white rat following partial surgical removal. *Arch Pathol (Chic)* 1931;12:186-202.
6. Greene AK, Puder M. Partial hepatectomy in the mouse: technique and perioperative management. *J Invest Surg* 2003;16:99-102.
7. Nikfarjam M, Malcontenti-Wilson C, Fanartzis M, et al. A model of partial hepatectomy in mice. *J Invest Surg* 2004;17:291-4.
8. Redaelli CA, Semela D, Carrick FE, et al. Effect of vascular endothelial growth factor on functional recovery after hepatectomy in lean and obese mice. *J Hepatol* 2004;40:305-12.
9. Pennisi PA, Kopchick JJ, Thorgeirsson S, et al. Role of growth hormone (GH) in liver regeneration. *Endocrinology* 2004;145:4748-55.
10. Strey CW, Winters MS, Markiewski MM, et al. Partial hepatectomy induced liver proteome changes in mice. *Proteomics* 2005;5:318-25.
11. Sato T, El-Assal ON, Ono T, et al. Sinusoidal endothelial cell proliferation and expression of angiopoietin/Tie family in regenerating rat liver. *J Hepatol* 2001;34:690-8.
12. Fausto N, Riehle KJ. Mechanisms of liver regeneration and their clinical implications. *J Hepatobiliary Pancreat Surg* 2005;12:181-9.
13. Bucher NL. Regeneration of mammalian liver. *Int Rev Cytol* 1963; 15:245-300.
14. Makino H, Togo S, Kubota T, et al. A good model of hepatic failure after excessive hepatectomy in mice. *J Surg Res* 2005;127:171-6.
15. Mitchell C, Nivison M, Jackson LF, et al. Heparin-binding epidermal growth factor-like growth factor links hepatocyte priming with cell cycle progression during liver regeneration. *J Biol Chem* 2005;280: 2562-8.
16. Guha C, Sharma A, Gupta S, et al. Amelioration of radiation-induced liver damage in partially hepatectomized rats by hepatocyte transplantation. *Cancer Res* 1999;59:5871-4.
17. Greene AK, Wiener S, Puder M, et al. Endothelial-directed hepatic regeneration after partial hepatectomy. *Ann Surg* 2003;237:530-5.
18. Sturm J, Keese M, Zhang H, et al. Liver regeneration in FGF-2-deficient mice: VEGF acts as potential functional substitute for FGF-2. *Liver Int* 2004;24:161-8.
19. Sturm JW, Zhang H, Magdeburg R, et al. Altered apoptotic response and different liver structure during liver regeneration in FGF-2-deficient mice. *Cell Physiol Biochem* 2004;14:249-60.
20. Martinez-Hernandez A, Amenta PS. The extracellular matrix in hepatic regeneration. *FASEB J* 1995;9:1401-10.
21. Hoffmann B, Paul D. Basic fibroblast growth factor and transforming

growth factor-alpha are hepatotrophic mitogens in vitro. *J Cell Physiol* 1990;142:149-54.

22. Presta M, Statuto M, Rusnati M, et al. Characterization of a Mr 25,000 basic fibroblast growth factor form in adult, regenerating, and fetal rat liver. *Biochem Biophys Res Commun* 1989;164:1182-9.
23. Hioki O, Minemura M, Shimizu Y, et al. Expression and localization of basic fibroblast growth factor (bFGF) in the repair process of rat liver injury. *J Hepatol* 1996;24:217-24.
24. Ma TY, Kikuchi M, Sarfeh IJ, et al. Basic fibroblast growth factor stimulates repair of wounded hepatocyte monolayer: modulatory role of protein kinase A and extracellular matrix. *J Lab Clin Med* 1999;134:363-71.
25. Shimizu A, Tada K, Shukunami C, et al. A novel alternatively spliced fibroblast growth factor receptor 3 isoform lacking the acid box domain is expressed during chondrogenic differentiation of ATDC5 cells. *J Biol Chem* 2001;276:11031-40.
26. Jung J, Zheng M, Goldfarb M, et al. Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* 1999;284:1998-2003.