Lovastatin decreases mortality and improves liver functions in fulminant hepatic failure from 90% partial hepatectomy in rats

Shi-Rong Cai¹, Kentaro Motoyama², Katherine J. Shen¹, Susan C. Kennedy¹, M. Wayne Flye² and Katherine Parker Ponder¹

Departments of Internal Medicine and Biochemistry and Molecular Biophysics, and Surgery, Washington University School of Medicine, USA

**Background/Aims:** Liver insufficiency occurs when the liver cannot perform critical functions such as ammonia metabolism, gluconeogenesis, or production of coagulation factors. The hypothesis of this study was that decreased function of existing hepatocytes may contribute to hepatic failure, and that the function of these cells might be increased pharmacologically. Lovastatin is a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor that inhibits cholesterol biosynthesis and affects the activity of some signal transduction pathways and liver transcription factors. Changes in hepatic transcription factors during liver regeneration might result in decreased liver functions, and lovastatin might prevent these changes.

**Methods:** Rats received 90% partial hepatectomy (90% PH), and either lovastatin or vehicle alone daily. Survival and liver functions were assessed.

**Results:** Lovastatin increased survival to 58% (vs. 6% in controls that received 90% PH without drug), decreased the peak ammonia level to 427 μM (vs. 846 μM in controls), increased the nadir of glucose to 88 mg/dl (vs. 57 mg/dl in controls), decreased the peak prothrombin time to 23 s (vs 29 s in controls), and decreased the peak activated partial thromboplastin time to 29 s (vs. 39 s in controls). The full survival and metabolic benefits were observed when lovastatin was started at 30 min after 90% PH, but lovastatin was less efficacious when started at later times.

**Conclusions:** Lovastatin increases the function of existing hepatocytes and might be used to improve liver function after extensive hepatic resection.

**Key words:** Ammonia; Coagulation; Glucose, Liver regeneration.

Liver insufficiency occurs when the liver cannot perform gluconeogenesis, ammonia or bilirubin metabolism, or synthesis of blood proteins. The clinical manifestations include hypoglycemia, encephalopathy, jaundice, and bleeding. Fulminant hepatic failure has an incidence of 17 per 100 000 with a survival rate of 10-30% (1). Treatment of fulminant hepatic failure involves supportive care to allow possible recovery of the liver. In severe cases, liver transplantation is required for survival. This is an expensive procedure that requires lifelong immunosuppression and is limited by the availability of livers.

Hepatic failure may result from an insufficient number of hepatocytes, inadequate function of the existing hepatocytes, or a combination of these effects. We previously showed that a human p21-K-ras gene whose protein product was constitutively active inhibited several liver-specific functions when it was expressed from a retroviral vector in hepatocytes of rats in vivo (2,3). This led us to hypothesize that the activation of p21-Ras or other signaling pathways that occurs during liver regeneration (4–9) might play a role in the liver insufficiency of hepatic failure, and that pharmacological inhibition of p21-Ras signaling might increase the function of the existing hepatocytes.

The biological effect of p21-Ras and other signal transduction pathways can be inhibited pharmacologically. Lovastatin is a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitor (10–12) that blocks the synthesis of intermediates of the cholesterol biosynthetic pathway that are added posttranslationally to p21-Ras and are necessary for it to transduce a signal. The administration of HMG-CoA reductase inhibitors decreased p21-Ras signaling and
slowed the growth of p21-Ras-dependent tumors in vivo (13–16). In addition, signal transduction via G-protein-coupled receptors such as the adrenergic and glucagon receptor can be affected by lovastatin (17). Finally, the activity of the transcription factors sterol-response element binding protein (SREBP) and the liver X receptor (LXR) can be increased (18) or decreased (19), respectively, by lovastatin.

A 90% PH model was used to test the effect of lovastatin in fulminant hepatic failure for five reasons: 1) this model does not involve a toxin; 2) death occurs within 48 h in the majority of rats (20–23); 3) metabolic insufficiency clearly plays an important role, as survival can be increased by administration of glucose (20–23); 4) p21-Ras and other signal transduction proteins are likely to be activated; and 5) partial hepatectomy can result in liver failure in humans with primary or metastatic liver cancer (24–27). We demonstrate here that lovastatin resulted in a striking improvement in glucose levels, ammonia levels, and coagulation tests, and increased the survival rate by 9-fold in this model.

Materials and Methods

90% PH
Sprague-Dawley rats weighing 260 to 320 g were housed for >1 week prior to surgery with 12-h light/dark cycles. They were fed standard rat chow and water ad libitum unless otherwise stated. Lovastatin (Merck & Co., Inc.) was dissolved at 7 mg/ml in a 1:1 mixture of PBS (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.4), polyethylene glycol and stored at -20°C. 20 mg/kg (about 3 ml) was given by gavage once per day. Rats were anesthetized with inhaled methoxyfluorane. For 90% PH, the median, left, right upper, and right lower lobes were removed, leaving the caudate lobes which represent 10–11% of the original liver mass (20). For 70% PH, the left and median lobes were removed. All surgeries were performed between 8 a.m. and noon. Individual animals were bled once per day by collecting 200 to 400 μl of blood from the retroorbital plexus. Blood was anticoagulated with 1/10 volume of 3.8% sodium citrate.

Statistical analyses
For determination of statistical significance of survival rates between different groups, the Lifetest log-rank procedure was used with software from statistical analysis system software (SAS, Inc., Cary, NC, USA). For comparison of liver function tests between lovastatin- and vehicle-treated rats, an unpaired two-tailed Student's t-test was performed at each time point using the program Instat from GraphPAD Software (San Diego, CA, USA). For comparison of liver function tests between several groups that started lovastatin at various times after 90% PH and controls, one-way analysis of variation (ANOVA) was performed using Instat.

Liver function tests
Blood glucose was determined using a glucometer from Medisense (Bedford, MA, USA) and Precision Q.L.D. test strips. Venous plasma ammonia (#171-C), total (direct and indirect) serum bilirubin (#605C), and serum glutamic pyruvic transaminase (SGPT)/alanine aminotransferase (ALT) (#505-P) levels were determined using kits from Sigma Chemical (St. Louis, MO, USA) that were adapted to allow quantitation on an ELISA reader.

Coagulation assays
Plasma was stored on ice and assayed the same day. For the prothrombin time (PT), 100 μl of plasma was mixed with 200 μl of 11.6 mM CaCl2 with rabbit brain thromboplastin (Sigma Chemical), and clot formation detected with a BBL fibrometer (Becton, Dickinson, and Company, Cockeysville, MD, USA). The activated partial thromboplastin time (aPTT) was determined using 100 μl of plasma, 100 μl of 25 mM CaCl2, and 100 μl of phospholipid with alumina coated silica particles.

5'-Bromo-2'-deoxyuridine (BrdU) labeling
BrdU was injected at 100 mg/kg i.p. Most animals were sacrificed 2 h later, although some animals received 3 doses of BrdU. The time of replication was reported as the interval between the initial injection and the time of sacrifice. BrdU staining of frozen liver sections was performed using a goat anti-BrdU antibody and the percentage of labeled cells determined as previously described (28).

Analysis of liver weights
The fractional liver weight is the mass of the liver divided by the total body mass. All animals were weighed prior to surgery or at sacrifice to determine the total body mass. For determination of liver weight prior to 90% PH, the weight of the removed liver was multiplied by 1.11 (assuming that the amount of liver removed was 90%).

Results
Survival and liver function tests when lovastatin was started 3 days prior to 90% PH and continued for 14 days after 90% PH
Because the time required for lovastatin to exert its effect upon signal transduction proteins or transcription factors was unclear, lovastatin was started 3 days before 90% PH and continued daily for 2 weeks after surgery in order to maximize the possibility of seeing an effect. The dose of lovastatin used (20 mg/kg/day) was similar to a dose that inhibited post-translational modification of p21-Ras (14,15) and inhibited the growth of p21-Ras-dependent tumors in rodents (13–15). The survival rate at 2 weeks in lovastatin-treated rats (56.2%) was 9-fold higher than in the controls that underwent 90% PH but did not receive lovastatin (6.3%; p=0.0039 using the log-rank test), as shown in Fig. 1.

The dramatic improvement in survival in lovastatin-treated rats, as compared with untreated rats, after 90% PH suggested that lovastatin improved liver functions in the former group. We had previously noted that activated p21-Ras inhibited the urea-synthetic enzyme ornithine transcarbamylase and the gluconeogenic enzyme glucose-6-phosphatase. We therefore predicted that lovastatin might improve ammonia and glucose levels. Venous plasma ammonia levels increased to a peak of 426.2 μM at 16 h after 90% PH for lovastatin-treated rats, which was 4-fold higher than the upper limit of normal using plasma collected in the same fashion (100 μM), as shown in Fig. 2A. However, in the controls that received 90% PH without drug, the ammonia levels were almost 2-fold higher than in the lovastatin-treated rats, and they fell more slowly there-
Effect of lovastatin in liver insufficiency

Fig. 1. Effect of lovastatin upon survival after 90% PH. Rats were treated with a daily dose of lovastatin (n=16) at 20 mgkg/day beginning 3 days prior to 90% PH and continuing for 14 days after 90% PH. On the day of 90% PH, the lovastatin was administered at 30 min after 90% PH. Controls rats (No drug; n=16) received 90% PH and vehicle at the same timepoints.

Fig. 2A. Effect of lovastatin upon glucose levels in animals without access to food. Although it is likely that improved glucose levels in the lovastatin-treated rats, as compared with the vehicle-treated rats, were due to improved output of glucose by the liver, an alternative explanation is that lovastatin-treated rats had increased oral intake. We therefore withhold food from some rats after 90% PH, and determined glucose levels, as shown in Fig. 3. Glucose levels at 6 and 10 h were 89 and 85 mg/dl, respectively, for rats that received lovastatin, which was higher than the values of 74 (p=0.0078) and 59 (p=0.0005), respectively, for rats that received vehicle alone. For both groups, glucose levels at 6 and 10 h were similar to those observed in rats that had free access to food (Fig. 2B). We conclude that differences in oral intake cannot account for the differences in blood glucose levels between the two groups during the first 10 h after 90% PH.

Fig. 2C. Effect of lovastatin when started at varying times after 90% PH. We tested how long one could wait to start lovastatin after performing a 90% PH and still observe a clinical effect, as some patients might receive emergency hepatic resection. When the first dose of lovastatin was administered at 30 min after 90% PH, the survival at 7 days was 61% (Fig. 4A). This was similar to the survival of 56% observed when lovastatin was started at 3 days prior to 90% PH, and was statistically higher than the survival rate of 10% observed in vehicle-treated rats (p-value=0.0013 with log-rank test). However, survival at 7 days decreased to 31%, 16% or 8% when lovastatin was started at 2, 4, or 8 h, respectively, after 90% PH, and none of these were statistically different from the vehicle-treated controls.

Fig. 2D. Effect of lovastatin upon coagulation. Coagulopathy contributes to morbidity and mortality in fulminant hepatic failure. Rats received 90% PH and

levels were similar to or lower than those in the control group.

Effect of lovastatin upon glucose levels in animals without access to food

Although it is likely that improved glucose levels in the lovastatin-treated rats, as compared with the vehicle-treated rats, were due to improved output of glucose by the liver, an alternative explanation is that lovastatin-treated rats had increased oral intake. We therefore withheld food from some rats after 90% PH, and determined glucose levels, as shown in Fig. 3. Glucose levels at 6 and 10 h were 89 and 85 mg/dl, respectively, for rats that received lovastatin, which was higher than the values of 74 (p=0.0078) and 59 (p=0.0005), respectively, for rats that received vehicle alone. For both groups, glucose levels at 6 and 10 h were similar to those observed in rats that had free access to food (Fig. 2B). We conclude that differences in oral intake cannot account for the differences in blood glucose levels between the two groups during the first 10 h after 90% PH.

Effect of lovastatin when started at varying times after 90% PH

We tested how long one could wait to start lovastatin after performing a 90% PH and still observe a clinical effect, as some patients might receive emergency hepatic resection. When the first dose of lovastatin was administered at 30 min after 90% PH, the survival at 7 days was 61% (Fig. 4A). This was similar to the survival of 56% observed when lovastatin was started at 3 days prior to 90% PH, and was statistically higher than the survival rate of 10% observed in vehicle-treated rats (p-value=0.0013 with log-rank test). However, survival at 7 days decreased to 31%, 16% or 8% when lovastatin was started at 2, 4, or 8 h, respectively, after 90% PH, and none of these were statistically different from the vehicle-treated controls.

Ammonia (Fig. 4B) and glucose (Fig. 4C) levels at 24 h after 90% PH were significantly better for the animals that started lovastatin at 30 min to 4 h after 90% PH than in control rats that did not receive lovastatin. Ammonia and glucose levels were not improved for rats that started lovastatin at 8 h after 90% PH. The total serum bilirubin level at 1 day after 90% PH in rats that started lovastatin at 30 min after 90% PH was significantly lower than for rats that did not receive drug (Fig. 4D).

Effect of lovastatin upon coagulation

Coagulopathy contributes to morbidity and mortality in fulminant hepatic failure. Rats received 90% PH and
Fig. 2. Effect of lovastatin upon liver function tests after 90% PH. Rats were treated with lovastatin or vehicle alone and 90% PH, as described in Fig. 1. The rats studied included those described in Fig. 1, as well as rats that received lovastatin or vehicle alone in the same fashion, but were sacrificed before or at 24 h after 90% PH in order to obtain liver tissue for analysis. For the data points shown at day 2 or later, blood was obtained from all survivors of the animals described in Fig. 1. All data were reported as the average ± standard error of the mean (SEM). The number of animals evaluated at each timepoint (N) is shown. A. Ammonia levels. Plasma was analyzed for ammonia levels. Normal ammonia levels in these rats (average ± standard deviations) prior to any sort of manipulation were 0 to 100 μM, as indicated by the shaded region. Levels in lovastatin-treated rats were compared with the levels in rats that did not receive drug at each timepoint after 90% PH for statistically significant differences using the Student's t-test. *Indicates a p-value between 0.05 and 0.005, ** a p-value between 0.005 and 0.0005, and *** a p-value <0.0005. B. Glucose levels. Normal glucose levels in rats prior to any sort of manipulation were 103 to 139 mg/dl. C. Total bilirubin levels. Normal bilirubin levels in rats prior to any sort of manipulation were 0 to 0.25 mg/dl. D. SGPT levels. Normal SGPT levels in rats prior to any sort of manipulation were 0 to 40 International Units (IU)/ml.

either lovastatin or vehicle alone, and were sacrificed in order to obtain blood for coagulation assays. Fig. 5 demonstrates that the PT and aPTT at 28 h after 90% PH were 23 and 29 s, respectively, for the lovastatin-treated rats. These were lower than the values of 29 (p<0.0001) and 39 (p=0.0003) s, respectively, for the vehicle-treated rats. We conclude that lovastatin has a dramatic effect upon coagulation parameters at 28 h after 90% PH.

Effect of lovastatin upon liver regeneration
Drugs that inhibit signal transduction might prevent liver regeneration. We therefore examined liver regeneration after 90% PH by analysis of liver weight.
Glucose
No Caloric Intake

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Hours after 90% PH
- No Drug
- Lovastatin

Fig. 3. Glucose levels in rats that had food withheld after 90% PH. Rats were treated with 90% PH and lovastatin or vehicle alone as described in the legend to Fig. 1. After 90% PH, food, but not water, was withheld for 24 h. Criteria for statistical significance are as described in Fig. 2.

and BrdU labeling. The average fractional liver weight just before 90% PH was 3.3% for controls or for rats that had received lovastatin for 3 days, which was similar to that in a previous report (30). For both groups, the liver represented 0.4% of the total body weight at 24 h after 90% PH, demonstrating that appreciable liver regeneration had not yet occurred. The average fractional liver weight in the lovastatin-treated rats was 3.0±0.07% and 3.1±0.05% at 7 and 14 days, respectively, after 90% PH. This was similar to the level observed in rats that did not receive drug at the same times after 90% PH. These data demonstrated that lovastatin did not prevent liver regeneration.

To assess the rate of liver regeneration, we determined the percentage of hepatocytes that were replicating after 70% or 90% PH in lovastatin- and vehicle-treated animals. After incorporation of the thymidine analog BrdU into their nuclei, dividing cells can be identified by immunostaining with an anti-BrdU antibody. We chose 70% PH for an initial study, as the time of replication after 70% PH is better defined than after 90% PH (31), and the high mortality rate in controls complicates comparison of the labeling index between the two groups after 90% PH. Rats that received vehicle alone had a BrdU labeling index of 31.1±1.7% (SEM; n=4) at 24–26 h after 70% PH (Fig. 6A), which was similar to the value reported previously (31). This value was statistically higher (p=0.036) than for rats that received lovastatin (22.45±1.9% SEM; n=2), as shown in Fig. 6B.

BrdU labeling was also determined in lovastatin-treated rats after 90% PH. The labeling index at 24–26 h was 5% (n=1), at 28–30 h was 2.1±1% (n=2), and at 32–34 h was 5% (n=1) (data not shown). When 3 doses of BrdU were injected between 32 and 44 h after 90% PH for one lovastatin-treated animal, 14% of the hepatocytes had replicated during that interval (Fig. 6C). All of the control rats that received 90% PH without drug died prior to injection of BrdU, so we could not determine the labeling index in the absence of lovastatin treatment. We conclude that lovastatin slowed but did not prevent hepatocyte replication at 24 h after 70% PH, and that replication rates were low but detectable within 44 h after 90% PH.

Lovastatin does not prevent hepatic steatosis after 90% PH
Both 90% PH (20,32,33) and activation of p21-Ras (2) result in microvesicular steatosis in hepatocytes, while 70% PH results in triglyceride (TG) accumulation in the liver (34). We therefore hypothesized that activation of p21-Ras or other signal transduction pathways might contribute to steatosis after 70% or 90% PH, and that lovastatin might prevent its occurrence. However, large vesicles were present in the majority of cells in a lovastatin-treated rat at 30 h after 90% PH (Fig. 6D), which was consistent with steatosis. Livers from untreated rats had a similar histological appearance at early times after 90% PH (data not shown). Oil red O stain of frozen liver sections and triglyceride analysis of liver extracts confirmed that there were large amounts of fat in both groups (data not shown) at early times. Steatosis had disappeared in lovastatin-treated rats by 7 days (Fig. 6E), and remained absent at 14 days after 90% PH. However, steatosis was still present at 7 days after 90% PH in the sole surviving rat that did not receive drug (Fig. 6F), although it had resolved by 14 days. We conclude that lovastatin does not prevent hepatic steatosis after 90% PH, although it may hasten its resolution.

Discussion
Fulminant hepatic failure can result from an insufficient number of hepatocytes, decreased functional activity in the existing hepatocytes, or a combination of these effects. These data demonstrate that lovastatin
Fig. 4. Effect ofLovastatin upon survival and metabolic function whenLovastatin was started at various times after 90% PH. Lovastatin at 20 mg/kg/day was started at 30 min after (30'; n=21), 2 h after (2 h; n=13), 4 h after (4 h; n=13), or 8 h after (8 h; n=13) 90% PH. Controls (C; n=10) received vehicle alone beginning at 30 min after 90% PH. All rats received a daily dose ofLovastatin or vehicle thereafter beginning at 24 h or sooner after the first dose. A. Survival. The legend for this panel is shown at the top left. The percent surviving animals at various times after 90% PH is shown for each group. B-D. Analysis of ammonia (panel B), glucose (panel C), and total bilirubin (panel D) after 90% PH. All data represent samples that were collected at 24 h after 90% PH. The time at whichLovastatin was initiated after 90% PH is indicated underneath each graph, while C indicates controls that started vehicle alone at 30 min after 90% PH. All data were reported as the average±SEM, normal values are indicated by the shaded region, and statistical analyses were between each group that receivedLovastatin and the group that received vehicle alone using ANOVA. *Indicates a Bonferroni p-value of 0.01 to 0.05; ** indicates a Bonferroni p-value of 0.001 to 0.01, and *** indicates a Bonferroni p-value<0.001.

improves survival, glucose levels, ammonia levels, and coagulation parameters after 90% PH. Since metabolic and coagulation parameters are improved prior to the initiation of liver regeneration, Lovastatin is acting to improve the functional activity of existing hepatocytes.

Lovastatin had a potent effect upon survival after 90% PH

The survival in rats that started Lovastatin before or shortly after 90% PH (~60%) was similar to the survival observed in rats that were supplemented with glu-
Ammonia levels after 90% PH were markedly improved with lovastatin

Ammonia has been implicated as an etiologic factor in hepatic encephalopathy for three reasons. First, ammonia levels correlate somewhat with the degree of mental impairment in humans (35). Second, administration of ammonia in rats (36) or humans (37) with liver insufficiency can induce encephalopathy, brain swelling, and sometimes death, while methods that decrease ammonia levels can improve symptoms. Third, patients with urea cycle disorders develop encephalopathy that correlates with ammonia levels (38,39). However, 10% of patients with hepatic encephalopathy have normal ammonia levels (35), and other intermediates of amino acid metabolism may contribute to neurological symptoms. Nevertheless, elevated ammonia levels are probably a valid indication of sufficient impairment in liver function to result in encephalopathy.

The ~50% decrease in ammonia levels in lovastatin-treated as compared with vehicle-treated rats may have contributed to the improved survival in the lovastatin-treated rats, although the improved glucose levels may have also played a role. A treatment that decreases ammonia levels should have an important clinical role in fulminant hepatic failure, as elevated ammonia is difficult to treat. We are currently investigating if genes involved in amino acid metabolism are induced by lovastatin after 90% PH.

Glucose levels after 90% PH were markedly improved with lovastatin

Hypoglycemia is an important indicator of liver insufficiency in humans. The improved glucose levels in the lovastatin-treated rats, as compared with the vehicle-treated controls, were likely due to increased hepatic output, as withholding food did not eliminate the differences between the two groups. Furthermore, preliminary data in a small number of animals demonstrate that hepatic glucose output is higher in the lovastatin-treated rats than in the vehicle-treated controls, and that both groups have low levels of peripheral glucose uptake. These data therefore suggest that lovastatin improved an important function of the liver, glucose production. Although improved glucose levels probably played a major role in the increase in survival in lovastatin-treated rats after 90% PH, this would be a less important clinical parameter to correct in humans, who can readily be supported with I.V. glucose during fulminant hepatic failure. Nevertheless, it indicates improvement in another liver function. We are currently
A. 70% (−): BrdU 24-26h

B. 70% (+): BrdU 24-26h

C. 90% (+): BrdU 32-44h

D. 90% (+): H&E 30h

E. 90% (+): H&E 7d

F. 90% (−): H&E 7d
testing if lovastatin alters the expression of genes that play a role in glycolysis or gluconeogenesis after 90% PH.

Coagulation assays after 90% PH were markedly improved with lovastatin

Coagulopathy is a major cause of morbidity and mortality in fulminant hepatic failure (1). The marked improvement in the PT and aPTT in lovastatin-treated as compared with vehicle-treated rats after 90% PH was probably due to increased production of coagulation factors. Improved coagulation would be an important clinical parameter to achieve, as the coagulopathy of fulminant hepatic failure is difficult to treat because of the short half-life of many coagulation factors such as Factor VII.

Lovastatin does not induce hepatocellular necrosis or prevent liver regeneration

Up to 2% of human patients who are treated with lovastatin for hypercholesterolemia develop liver enzyme levels that are >3-fold higher than the upper limit of normal, and lovastatin is considered to be contraindicated in humans with liver disease (11,12). Since the dose of lovastatin of 20 mg/kg/day used here was ~13-fold higher than the maximum dose of 1.6 mg/kg/day that is recommended for humans (29), hepatocellular necrosis could occur. However, the SGPT levels were similar for vehicle- and lovastatin-treated animals, and were very similar to that reported in a previous study after 90% PH (33). The moderate increase in SGPT may be due to hepatic steatosis or to transient ischemia at the time of the surgical procedure. Muscle necrosis can also occur in patients who are treated with lovastatin. However, creatine phosphokinase (CPK) levels were normal at all times after 90% PH for lovastatin-treated and control rats (data not shown).

A potential adverse effect of a drug that inhibits signal transduction pathways is that liver regeneration might be delayed or prevented. Indeed, the percentage of replicating cells at 24 h after 70% PH for lovastatin-treated rats was only 72% of the value observed in vehicle-treated rats. Furthermore, the percentage of replicating cells that we observed in this study in lovastatin-treated rats after 90% PH was lower than that observed by other investigators after 90% PH for glucose-treated rats that did not receive lovastatin (21,22). Although lovastatin delays hepatocyte replication after both 70% and 90% PH, significant numbers of BrdU-labeled cells were present. Furthermore, the liver had regenerated to almost normal size by 7 days after 90% PH. We conclude that lovastatin slows but does not prevent liver regeneration.

Mechanism of action of lovastatin after 90% PH

One critical question that is not addressed here concerns the biochemical mechanism by which lovastatin improves survival and liver function in this model of fulminant hepatic failure. Our original hypothesis was that lovastatin would inhibit p21-Ras prenylation, which would block its ability to transduce a signal. Alternatively, lovastatin may be inhibiting signal transduction via G-protein-coupled receptors, or by affecting the activity of transcription factors such as SREBP and LXRα. Studies to identify the pathway(s) by which lovastatin exerts its effect are in progress.

Fig. 6. Bromodeoxyuridine (BrdU) labeling and liver histology after 70% or 90% PH. Rats were treated with lovastatin at 20 mg/kg/day or with vehicle alone beginning at 3 days prior to 70% or 90% PH and continuing daily thereafter. A–B. BrdU labeling at 24–26 h after 70% PH. At 24 h after 70% PH, rats were injected with BrdU. The livers were harvested 2 h later, and immunostaining was performed on liver sections to identify cells that contained BrdU in their nucleus and therefore had recently replicated. The slides were counterstained with eosin. Control rats that received BrdU but did not receive 70% PH (or any other treatment) had very few labeled cells (data not shown), as reported previously (28). A. BrdU staining at 24–26 h after 70% PH for an untreated (−) control. Labeled nuclei (indicated by an arrow) are more prominent near the portal vein (PV) and were more prominent near the portal vein (PV), although the percentage (14%±2.1%) was lower than after 70% PH. 20X. D. Hematoxylin and eosin (H&E) staining at 30 h after 90% PH for a lovastatin-treated (+) rat. The liver was fixed with formalin, and a paraffin-embedded section was stained with H&E. Large vacuoles (indicated by an arrow) were present within the hepatocytes, which was consistent with hepatic steatosis. 40X. E. H&E staining at 7 days after 90% PH for a lovastatin-treated (+) rat. The steatosis observed earlier had resolved. 40X. F. H&E staining at 7 days after 90% PH for an untreated (−) rat. Steatosis is still present, as indicated by the arrow. 40X.
Potential implications for humans with fulminating hepatic failure

This study demonstrates that lovastatin can improve the function of existing hepatocytes in fulminant hepatic failure induced by 90% PH in rats. It is possible that it might be useful in humans with hepatic failure due to liver resection for trauma, or the treatment of primary or metastatic liver cancer (24–27). However, this experimental result will need to be confirmed in larger animals, whose livers regenerate more slowly than rats (40,41). Furthermore, lovastatin may fail to improve liver functions after liver resection when underlying liver disease is present. Finally, additional dose optimization will need to be performed as the dose of lovastatin used here was 13-fold higher than that recommended for humans with hypercholesterolemia.

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