Rosuvastatin potentiates pro-angiogenesis and enhanced liver regeneration in a rat model of massive hepatectomy

Chen-Fuh Lam, MD, PhD

Department of Anesthesiology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan

INTRODUCTION. The liver is an organ with high capability to regenerate following extensive tissue damage. The remnant hepatocytes are able to fully regenerate the liver back to its normal size. The hepatocytes reenter the proliferative cell cycle with increased DNA synthesis within 24 after hepatic injury. Resection of liver lobes (partial hepatectomy, PH) is the standard surgical approach in removal of macroscopic liver parenchymal lesions and transplantation of liver lobes is the ultimate therapy to cure the end-stage liver disease. However, hepatic failure can happen after PH or liver transplant with extremely high mortality up to 75%. Derangement of liver function or development of nonregenerative liver after massive PH or liver transplant are characterized by increased hepatocyte apoptosis and diminished liver regeneration due to ischemia reperfusion injury and prolonged inflammatory reaction, particularly in the presence of liver parenchymal diseases. Statins are the most commonly used of lipid lowering agents and they mediate several important pleiotropic effects in the improvement of vascular endothelial dysfunction, attenuation of inflammatory responses, and enhancement of tissue regeneration. This study tested the perioperative protective effect of a water soluble statin rosuvastatin in the liver regeneration after massive PH in rats.

METHODS. Sprague-Dawley rats were randomly assigned to receive placebo or rosuvastatin (15 mg/kg) in chow for 2 weeks. Extensive hepatectomy (70% PH) was performed one week after start of rosuvastatin treatment. Postoperative liver mass was assessed by the liver growth ratio normalized by body mass. Serum levels of liver enzymes and inflammatory cytokines were determined. Expressions of liver regenerative biomarkers were analyzed using Western blotting and immunostaining. The regenerated liver tissues were examined under microscopy.

RESULTS. Hepatectomy was successfully performed in rats with similar perioperative mortality (Table 1) and rosuvastatin reduced serum concentration of aspartarte aminotransferase at seven days after PH (Table 2). Liver growth ratio was significantly increased in the rats treated with rosuvastatin (0.59±0.09 vs 0.72±0.10, P=0.02; Table 1 and Figure 1). Administration of rosuvastatin reduced serum level of monocyte chemoattractant protein (MCP)-1 and increased IL-6 level in the remnant liver tissue (Figure 2). Protein expressions of liver regenerative markers (VEGFR2, HGF, IL-6 and PCNA) were significantly upregulated after PH, indicating the ongoing process of liver regeneration. (Figure 3). Interestingly, the expressions of these biomarkers were more significantly enhanced in the rosuvastatin group (Figure 3). Immunohistochemical analysis confirmed the increased expression of PCNA+ hepatocytes in these animals at 5 and 7 days after operation (Figure 4). The micro-architectures of the regenerated liver were similar between the two treatment groups, but more foci of necrosis and increased sinusoidal infiltration of mononuclear cells were observed in the placebo-treated rats (Figure 5).

	Rosuvastatin		Control	
	Pre-PH	Post-PH	Pre-PH	Post-PH
Body weight (kg)	0.32±0.02	0.39±0.09	0.32±0.10	0.40±0.03
Total liver mass (g)	13.1±1.5	11.4±1.5	14.0±1.3	10.1±1.5
LBMR (mg/kg)	40.7±4.8	29.3±4.0	43.2±3.1	25.4±3.3
Liver growth ratio	0.72±0.10		0.59±0.09*	
Mortality	1/8		2/10	

Table 1. Changes in physiological parameters and liver growth ratio after 70% PH. LBMR: liver-to-body mass ratio. Data are presented as mean±SD. *P= 0.02, n=7-8 different animals in each group.

	Rosuvastatin	Control
AST (U/L)	90±6.9	105±7.4*
ALT (U/L)	50±4.3	54±5.1
Albumin (U/L)	2.6±0.2	2.5±0.2
ALK-P (U/L)	355±16	358±35
Glucose (mg/dl)	163±6.7	168±17
Triglyceride (mg/dl)	54±4.3	72±19
Cholesterol (mg/dl)	50±5.4	47±4.8
HDL (mg/dl)	23±1.9	19±1.3*
CRP (mg/L)	3.7±0.1	3.6±0.2

Table 2. Serum biochemistry tests at 1 week after PH. Data are presented as mean±SD. *P= 0.02, n=7-8.

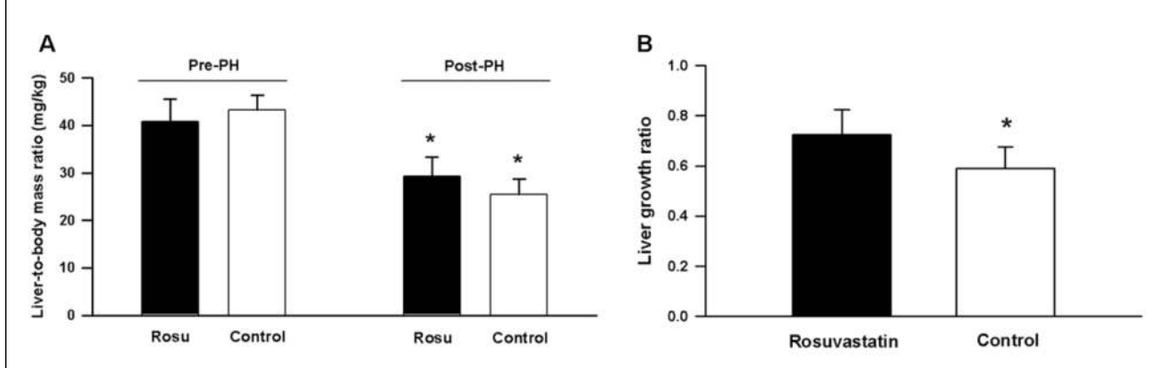

Figure 1. The regeneration of remnant liver was assessed by the liver growth ratio. Liver-to-body mass ratio before and after partial hepatectomy (PH) were analyzed by 2-way ANOVA. *P< 0.05 vs baselines. Liver growth ratio was analyzed by unpaired t-test. *P= 0.02, n=7-8.

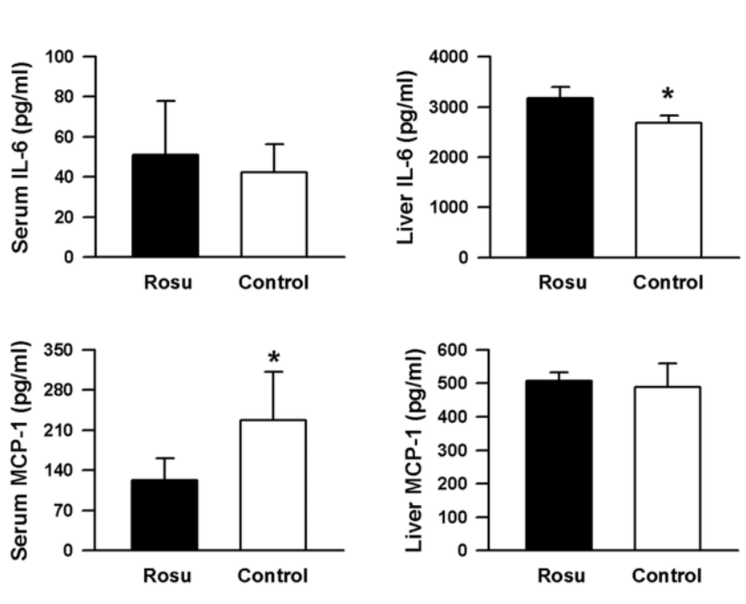

Figure 2. Serum and tissue levels of Inflammatory cytokines were determined using ELISA kits. Interleukin (IL)-6 is an important cytokine attributes to induction of the acute phase response to liver regeneration. IL-6 was significantly increased in the regenerated liver tissue of rosuvastatin-treated rats. Rosuvastatin also signigicantly suppressed the serum levels of MCP-1. Data were analyzed by unpaired t-test. *P<0.05, n=5-6.

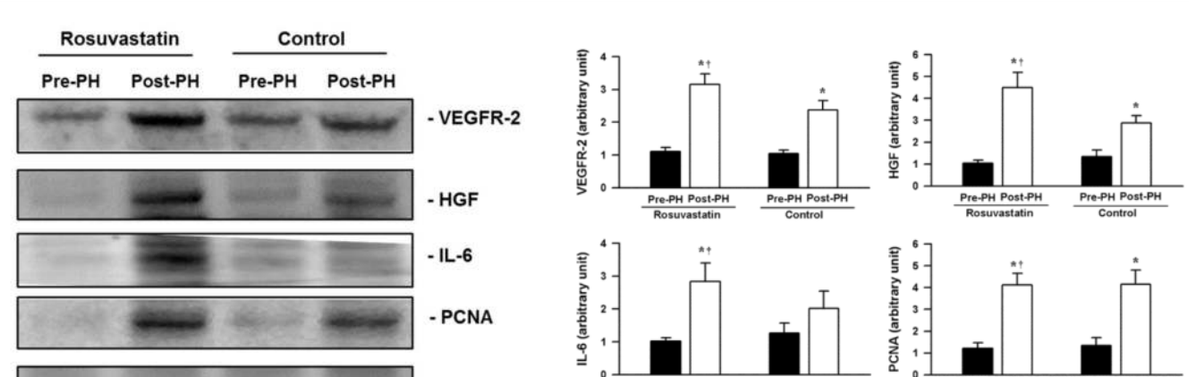

Figure 3. Expressions of markers for liver regeneration before (pre-PH) and after (post-PH) hepatectomy. These markers were enhanced after PH, and were more significantly upregulated in the rats treated with rosustatin. Data were analyzed by RM ANOVA, *P< 0.05 vs Pre-PH, †P< 0.05 vs control. n= 5.

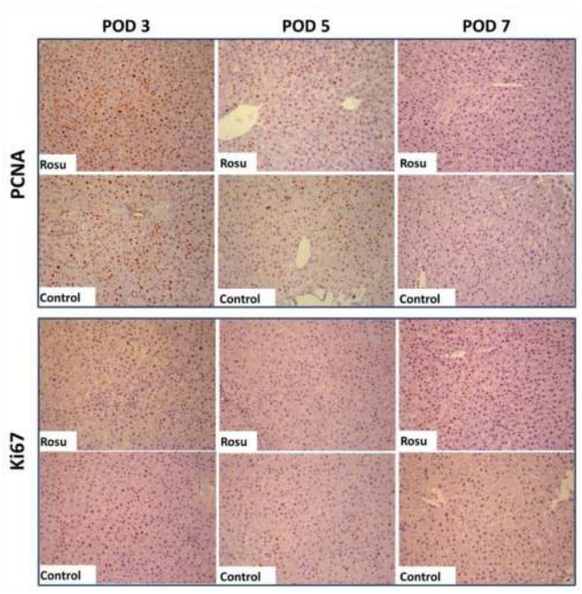

Figure 4. Tissue expressions of proliferating cell nuclear antigen (PCNA) and Ki67 in the regenerated remnant liver at postoperative days (POD) 3, 5 and 7.

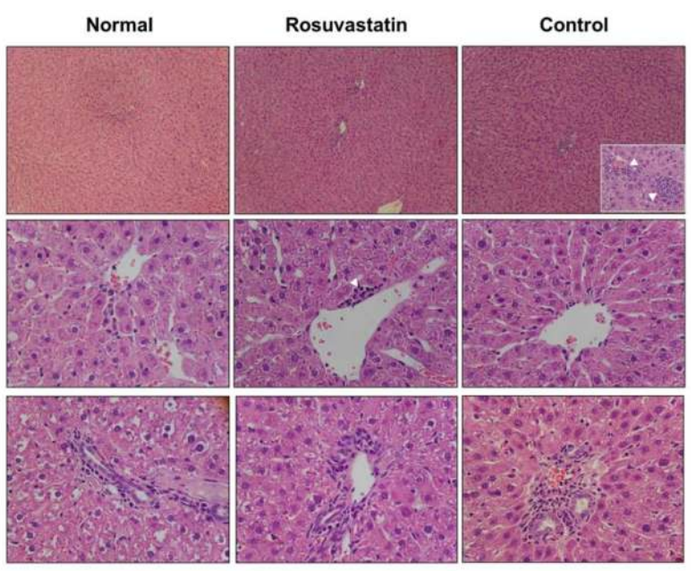

Figure 5. Representative H&E histological sections of liver in the naïve, rosuvastatin-treated and control rats. More spotty focal necrosis (arrowheads) with increased mononuclear cells infiltration in the sinusoids were observed in the liver parenchyma of control animals.
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CONCLUSION

Our study underscores that treatment with rosuvastatin attenuated the systemic inflammatory reaction and enhanced the regenerative response in the remnant liver by enhancement of tissue angiogenesis and proliferation of hepatocytes. The release of hepatocyte priming cytokine IL-6 and activation of cell proliferating mediators generates a microenvironment that is beneficial for liver regeneration. We suggest that perioperative use of statins may serve a pharmacologic therapeutic to improve hepatic regeneration, particularly in parenchymal liver diseases such as steatosis.

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Contact. Chen-Fuh Lam, MD, PhD
lamcf@mail.tcu.edu.tw