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RESEARCH PAPER

Simvastatin protects against cholestasis-induced liver injury

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Background: Bile duct obstruction is associated with hepatic accumulation of leukocytes and liver injury. The aim of this study was to evaluate the effect of simvastatin on cholestasis-induced liver inflammation and tissue damage.
Experimental approach: C57BL/6 mice were treated with simvastatin (0.02 and 0.2 mg·kg^{-1}) and vehicle before and after undergoing bile duct ligation (BDL) for 12 h. Leukocyte recruitment and microvascular perfusion in the liver were analysed using intravital fluorescence microscopy. CXC chemokines in the liver were determined by enzyme-linked immunosorbent assay. Liver damage was monitored by measuring serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Hepatic levels of myeloperoxidase (MPO) were also determined.
Key results: Administration of 0.2 mg·kg^{-1} simvastatin decreased ALT and AST by 87% and 83%, respectively, in BDL mice. This dose of simvastatin reduced hepatic formation of CXC chemokines by 37–82% and restored sinusoidal perfusion in cholestatic animals. Moreover, BDL-induced leukocyte adhesion in sinusoids and postsinusoidal venules, as well as MPO levels in the liver, was significantly reduced by simvastatin. Notably, administration of 0.2 mg·kg^{-1} simvastatin 2 h after BDL induction also decreased cholestatic liver injury and inflammation.
Conclusions and implications: These findings show that simvastatin protects against BDL-induced liver injury. The hepatoprotective effect of simvastatin is mediated, at least in part, by reduced formation of CXC chemokines and leukocyte recruitment. Thus, our novel data suggest that the use of statins may be an effective strategy to protect against the hepatic injury associated with obstructive jaundice.
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Keywords: adhesion; cholestasis; intravital fluorescence microscopy; leukocyte; sepsis

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDL, bile duct ligation; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; KC, cytokine-induced neutrophil chemoattractant; MIP-2, macrophage inflammatory protein-2; MPO, myeloperoxidase; PBS, phosphate-buffered saline

Introduction

Obstruction of the common bile duct or its tributaries is associated with liver damage and increased susceptibility to subsequent bacterial infections. Surgical and endoscopic decompression constitutes the main therapeutic options in patients with biliary obstruction but they may not be sufficient to prevent development of hepatic injury and septic complications. Thus, mechanistic studies are needed to delineate the pathophysiology of cholestasis-induced liver damage. The pathogenesis of cholestatic liver injury remains elusive although retained bile acids are thought to be a key feature in obstructive hepatocellular damage (Marschall *et al.*, 2006; Stedman *et al.*, 2006). Interestingly, recent studies have suggested that hepatic recruitment of leukocytes is of great importance in mediating cholestatic liver injury (Gujral *et al.*, 2003; 2004). The leukocyte extravasation process in the liver is complex and takes place in both sinusoids and postsinusoidal venules. While selectin-independent trapping of leukocytes may occur in sinusoids (Wong *et al.*, 1997), P-selectin-dependent rolling appears to be a critical component in leukocyte accumulation in postsinusoidal venules (Klintman *et al.*, 2002; 2004; Laschke *et al.*, 2007). Nonetheless, a common feature of leukocyte extravasation in both sinusoids and postsinusoidal venules is the key role of lymphocyte function antigen-1 in supporting firm adhesion to activated endothelial cells (Li *et al.*, 2004a). Moreover, it has been shown that hepatic formation of CXC

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chemokines, macrophage inflammatory protein-2 (MIP-2) and cytokine-induced neutrophil chemoattractant (KC) is critical for the extravasation of leukocytes in endotoxaemic liver injury (Li *et al.*, 2004b).

Statins are mainly used to regulate cholesterol synthesis in patients with increased risk of cardiovascular complications via inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase (Corsini *et al.*, 1995). However, there are accumulating data in the literature suggesting that statins, such as simvastatin, may also exert anti-inflammatory effects, such as inhibition of cytokine formation, adhesion molecule expression as well as reduction of nitric oxide production (Weber *et al.*, 1997; Giusti-Paiva *et al.*, 2004; Terblanche *et al.*, 2007); all of which could be of value in protecting against pathological inflammation and tissue damage. For example, previous studies have shown that simvastatin protects against tissue damage in models of sepsis (Merx *et al.*, 2004), ischaemia-reperfusion (Lefer *et al.*, 1999), glomerulonephritis (Christensen *et al.*, 2006) and asthma (McKay *et al.*, 2004). Nonetheless, the effect of simvastatin on cholestatic liver inflammation and damage is not known.

Based on the above findings, we investigated the effect of simvastatin on microvascular perfusion, leukocyte recruitment, CXC chemokine formation and hepatocellular injury in a model based on ligation of the common bile duct in mice. Moreover, the therapeutic potential of simvastatin given after bile duct ligation (BDL) induction was also determined.

Methods

Animals

Adult male C57BL/6 mice (21–27 g) were used for the study. The animals were housed one per cage on a 12 h light–12 h dark cycle and had free access to standard pellet food and tap water throughout the experiment. Animals were anaesthetized by i.p. administration of 7.5 mg ketamine hydrochloride and 2.5 mg xylazine 100 g^{-1} body weight. All experiments were approved by the local ethics committee at Lund University.

Experimental protocol

In anaesthetized animals, the common bile duct was prepared and carefully ligated with a 6-0 Prolene suture. Sham-operated animals received an i.p. injection of phosphate-buffered saline (PBS) and underwent an identical laparotomy and liver manipulation without BDL. Simvastatin (0.02 and 0.2 mg·kg^{-1} i.p.) and vehicle were given 10 min prior to laparotomy and BDL. In separate experiments, animals were treated with simvastatin (0.2 mg·kg^{-1}) 2 h after induction of BDL in order to examine the therapeutic potential of this strategy.

Intravital fluorescence microscopy

Twelve hours after BDL, analysis of hepatic microcirculation was performed by means of intravital fluorescence microscopy. For this purpose, a transverse subcostal incision was performed and the mice were positioned on their left side and the left liver lobe was carefully exteriorized for microscopic analysis. For intravital fluorescence microscopy, we used a modified Olympus microscope. The microscopic images were recorded by a charge-coupled device video camera and evaluated off-line. Blood perfusion within individual microvessels was studied after i.v. injection of 0.1 mL 5% fluorescein isothiocyanate (FITC)-labelled dextran 150 000 (contrast enhancement by intravascular staining of plasma). *In vivo* labelling of leukocytes with 0.1% rhodamine-6G (0.1 mL i.v.) enabled quantitative analysis of leukocyte flow behaviour in both sinusoids and postsinusoidal venules. Five postsinusoidal venules with connecting sinusoids were evaluated in each animal. Microcirculatory analysis included determination of sinusoidal perfusion by measuring the number of non-perfused sinusoids given as a percentage of the total number of sinusoids observed. Leukocyte rolling was measured by counting the number of cells rolling along the endothelium in postsinusoidal venules for 20 s and is expressed as cells min^{-1}. Leukocyte adhesion in sinusoids and postsinusoidal venules was quantified by counting the number of cells that remained stationary during the observation period of 20 s and is expressed as cells per 10 high power field and cells mm^{-2} of endothelial surface respectively. After intravital microscopic observations, animals were killed and blood was drawn from the inferior vena cava for analysis of bilirubin and liver enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), using standard spectrophotometric procedures.

Myeloperoxidase (MPO) activity

Liver tissue was collected, weighed and homogenized in 10 mL 0.5% hexadecyltrimethylammonium bromide. Subsequently, the sample was freeze-thawed, after which the MPO activity of the supernatant was assessed. The MPO activity was determined spectrophotometrically as the MPO-catalysed change in absorbance occurring in the redox reaction of H_2O_2 (460 nm, 25°C). Values are expressed as MPO u·g^{-1} liver tissue.

Enzyme-linked immunosorbent assay (ELISA)

The right liver lobe was weighed, washed and homogenized in PBS containing 1% penicillin and streptomycin and fungizone (100 u·mL^{-1}) and then kept cool in cold serum-free Dulbecco's modified Eagle's medium. After centrifugation, supernatants were collected and stored at –20°C until analysis of CXC chemokines, including MIP-2 and KC, by the use of double antibody Quantikine ELISA kits using recombinant murine KC and MIP-2 as standards.

Histology

Tissue samples were taken from the left liver lobe and fixed in 4% formaldehyde phosphate buffer over night. Dehydrated, paraffin-embedded, 6 µm sections were stained with hematoxylin and eosin and analysed under light microscopy.

Materials

Ketamine hydrochloride was obtained from Hoffman-La Roche (Basel, Switzerland); xylazine, Janssen Pharmaceutica

(Beerse, Belgium); simvastatin, Sigma-Aldrich (Stockholm, Sweden). The Olympus microscope (BX50WI) was from Olympus Optical Co. (GmbH, Hamburg, Germany). FITC-labelled dextran 150 000 and rhodamine-6G were from Sigma Chemical Co. (St. Louis, MO, USA); Quantikine ELISA kits, R & D Systems Europe (Abingdon, Oxon, UK).

Statistics

All data are presented as mean values ± s.e.mean. Statistical evaluations were performed using Kruskal-Wallis one-way analysis of variance on ranks followed by multiple comparisons *versus* control group (Dunn's method) (SigmaStat; Jandel Corporation, San Rafael, CA, USA). Statistical significance was considered for a value of $P < 0.05$.

Results

Hepatocellular damage

Ligation of the common bile duct significantly increased systemic bilirubin levels by more than threefold, suggesting that clear-cut cholestasis was induced in this model (Figure 1A). Bilirubin levels in mice pretreated with simvastatin were not different from that in vehicle-treated animals after BDL, indicating that the degree of cholestasis was similar in all experimental groups (Figure 1A). BDL caused substantial hepatocellular injury indicated by a more than 126-fold increase in liver enzymes (Figure 1B,C; $P < 0.05$ vs. Sham, $n = 5$). Pretreatment with simvastatin significantly reduced BDL-induced liver damage (Figure 1B,C). For example, administration of 0.2 mg·kg^{-1} of simvastatin reduced ALT and AST

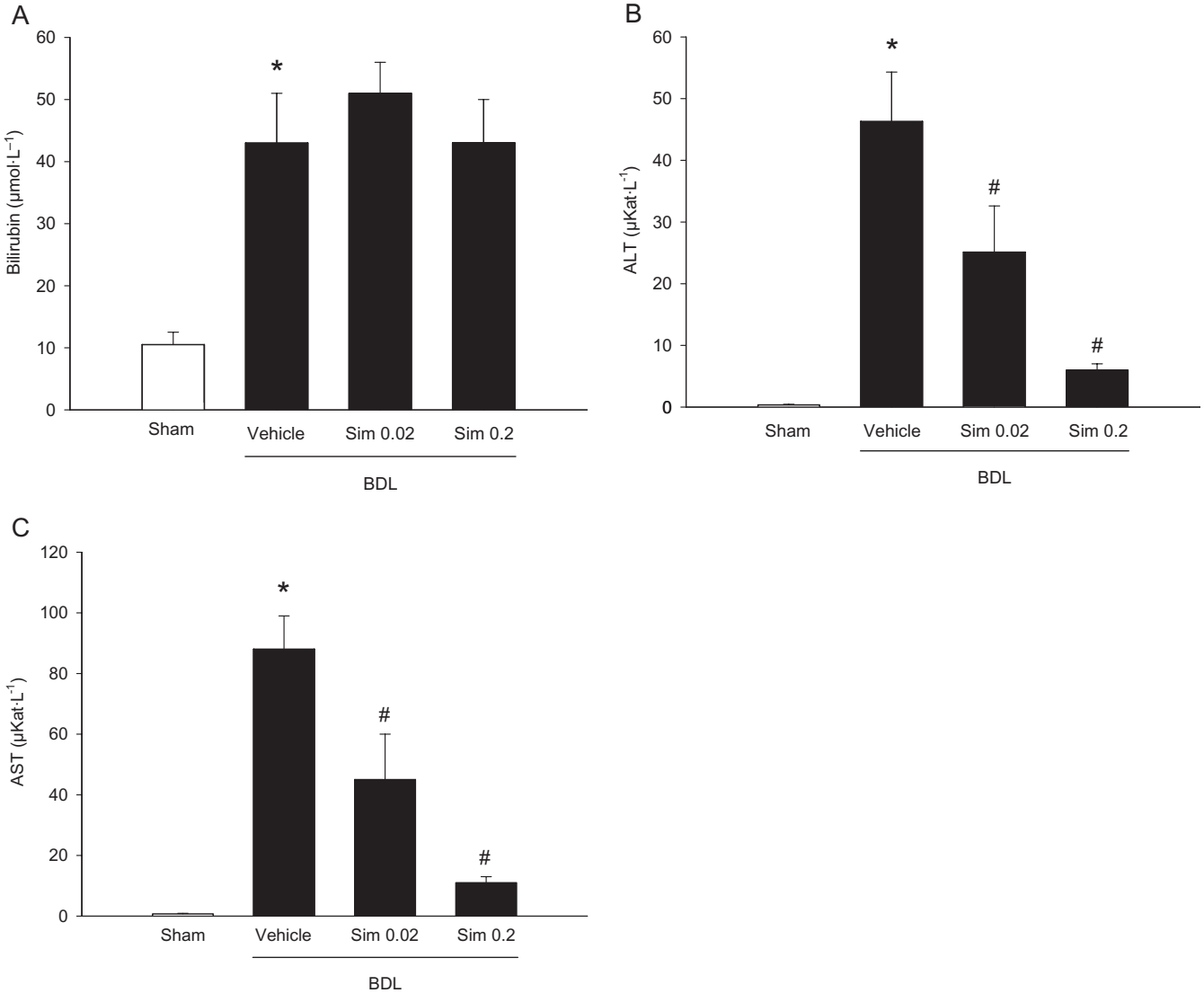

Figure 1 Bilirubin and liver enzymes 12 h after ligation of the common bile duct. Mice were treated with vehicle and simvastatin (Sim, 0.2 and 0.02 mg·kg^{-1}, i.p.) prior to bile duct ligation (BDL). Sham animals received only PBS. The levels of (A) bilirubin, (B) alanine aminotransferase (ALT) and (C) aspartate aminotransferase (AST) were determined spectrophotometrically. Data represent means ± s.e.mean, $n = 5$. *$P < 0.05$ versus Sham and $^{\#}P < 0.05$ versus vehicle + BDL. PBS, phosphate-buffered saline.

levels by 87% and 83%, respectively, in BDL mice (Figure 1B,C; $P < 0.05$ vs. vehicle+BDL, $n = 5$). Haematoxylin and eosin stained liver sections of sham-operated controls exhibited a normal hepatic architecture (Figure 2A), whereas marked destruction of the liver tissue with broad areas of necrosis was observed in cholestatic mice (Figure 2B). Pre-treatment with 0.2 mg·kg^{-1} simvastatin clearly reduced this cholestatic liver damage (Figure 2C).

Microvascular recruitment of leukocytes and perfusion

Having observed that simvastatin inhibits hepatic cholestatic liver injury, we next examined the effects of simvastatin on leukocyte-endothelium interactions in cholestatic mice in more detail by use of intravital fluorescence microscopy. It was observed that BDL increased leukocyte rolling and adhesion in postsinusoidal venules as well as adhesion in sinusoids (Figure 3, $P < 0.05$ vs. Sham, $n = 5$). BDL-induced leukocyte rolling in postsinusoidal venules was not different in any of the experimental groups (Figure 3A). In contrast, pre-treatment with 0.2 mg·kg^{-1} of simvastatin decreased BDL-induced leukocyte adhesion in the hepatic postsinusoidal venules by 73% (Figure 3B, $P < 0.05$ vs. vehicle+BDL, $n = 5$). Moreover, simvastatin significantly inhibited BDL-induced leukocyte adhesion in sinusoids by 27% (Figure 3C, $P < 0.05$ vs. vehicle+BDL, $n = 5$). Hepatic levels of MPO were used to determine the global accumulation of neutrophils, which constitute the predominant leukocyte subset in the liver early after BDL induction (Georgiev *et al.*, 2008). It was found that BDL increased MPO levels by more than threefold compared with sham mice (Figure 3D, $P < 0.05$ vs. Sham, $n = 5$). Administration of 0.2 mg·kg^{-1} of simvastatin significantly reduced the hepatic levels of MPO in BDL mice by 67% (Figure 3D, $P < 0.05$ vs. vehicle+BDL, $n = 5$). Representative photos of leukocyte-endothelium interactions in the hepatic microcirculation are shown in Figure 4. In addition, the percentage of non-perfused sinusoids was increased in BDL mice (Figure 5, $P < 0.05$ vs. Sham, $n = 5$) but this was decreased in the simvastatin-treated mice (Figure 5, $P < 0.05$ vs. vehicle+BDL, $n = 5$).

CXC chemokines

Hepatic levels of CXC chemokines in control animals were low but detectable (Figure 6, $n = 5$). Ligation of the common bile duct increased hepatic levels of MIP-2 and KC significantly (Figure 6, $P < 0.05$ vs. Sham, $n = 5$). Pre-treatment with 0.2 mg·kg^{-1} simvastatin decreased BDL-induced formation of MIP-2 and KC (Figure 6, $P < 0.05$ vs. vehicle+BDL, $n = 5$). Thus, simvastatin attenuated formation of MIP-2 and KC by 82% and 37%, respectively, in cholestatic mice.

Therapeutic effect of simvastatin

In order to test the therapeutic potential of simvastatin on cholestatic liver injury, separate mice were treated with 0.2 mg·kg^{-1} simvastatin 2 h after BDL induction. Indeed, we found that simvastatin decreased cholestasis-induced ALT and AST levels when given after BDL induction by more than 76% (Table 1, $P < 0.05$ vs. BDL+vehicle, $n = 5$). Moreover, late simvastatin treatment also significantly reduced BDL-induced

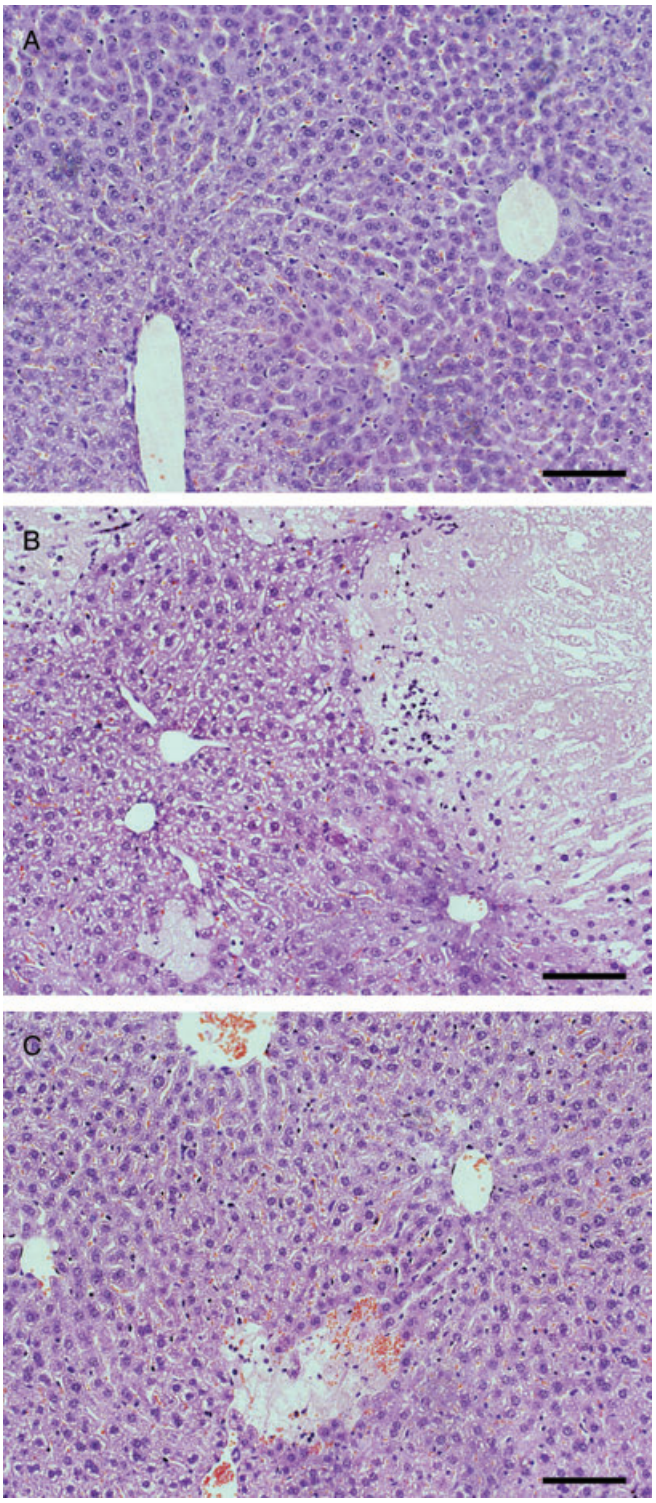

Figure 2 Haematoxylin and eosin-stained cross-sections of liver tissue. Sham animals received only PBS (A). Mice were injected (i.p.) with vehicle (B) and simvastatin (C) prior to bile duct ligation (BDL). Note that sham-operated mice exhibited a normal hepatic structure (A), whereas BDL in vehicle-treated animals resulted in a severe destruction of the liver with broad areas of necrosis (B). In contrast, pretreatment with 0.2 mg·kg^{-1} simvastatin decreased cholestatic liver damage (C). Scale bars: 100 μm. PBS, phosphate-buffered saline.

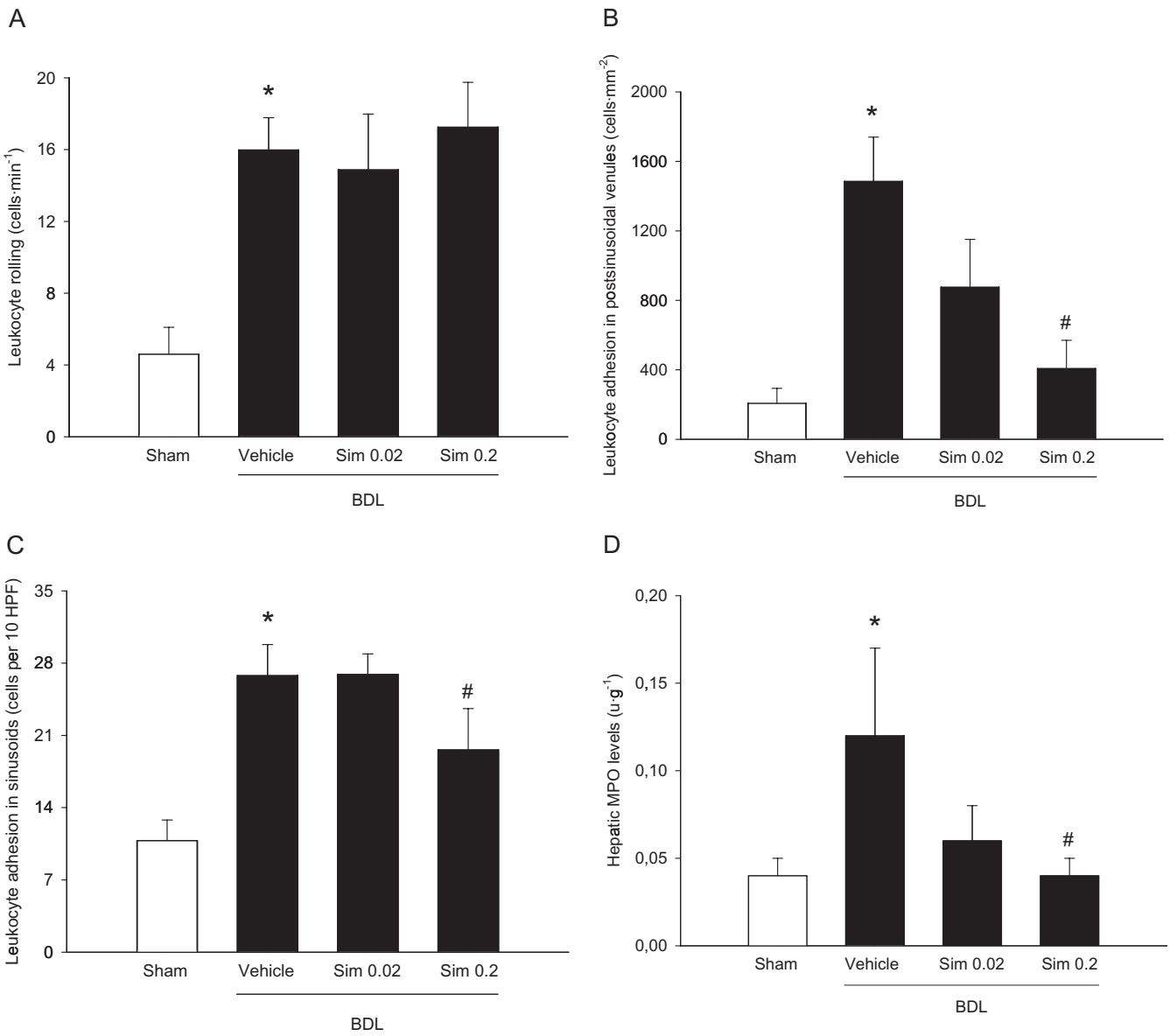

Figure 3 Leukocyte rolling (A) and adhesion in postsinusoidal venules (B) and leukocyte adhesion in sinusoids (C) and levels of MPO (D) 12 h after ligation of the common bile duct. Mice were treated with vehicle and simvastatin (Sim, 0.2 and 0.02 mg·kg^{-1}, i.p.) prior to bile duct ligation (BDL). Sham animals received only PBS. Data represent means ± s.e.mean, $n = 5$. *$P < 0.05$ versus Sham and $^{\#}P < 0.05$ versus vehicle + BDL. MPO, myeloperoxidase; PBS, phosphate-buffered saline.

formation of KC and MPO activity in the liver (Table 1, $P < 0.05$ vs. BDL + vehicle, $n = 5$), suggesting a therapeutic potential of simvastatin in cholestatic liver damage.

Discussion

Decompression of bile duct obstruction is commonly achieved by invasive procedures, which, however, may not be sufficient to avoid hepatocellular damage and septic complications. Thus, novel additional treatment options are needed to prevent cholestasis-induced perfusion failure and liver injury. In the present study, we showed that administration of simvastatin protects against hepatic injury in cholestatic mice. Our data demonstrate that simvastatin attenuates CXC chemokine formation and leukocyte recruitment in the liver of cholestatic mice. Moreover, these findings also indicate that simvastatin may exert a therapeutic effect on liver damage even when given after induction of cholestasis.

Several investigations have demonstrated that statins exert potent and pleiotropic anti-inflammatory effects as well as reducing cholesterol levels (Terblanche *et al.*, 2007). Herein, we demonstrate that simvastatin protects against the hepatic damage associated with bile duct obstruction. In fact, simvastatin decreased BDL-induced increases in liver enzymes by more than 83% in bile duct ligated mice. Notably, we also observed that simvastatin given 2 h after BDL induction reduced serum activities of ALT and AST by more than 70% as well as hepatic levels of MPO and KC by more than 38%, suggesting also a therapeutic potential of simvastatin in

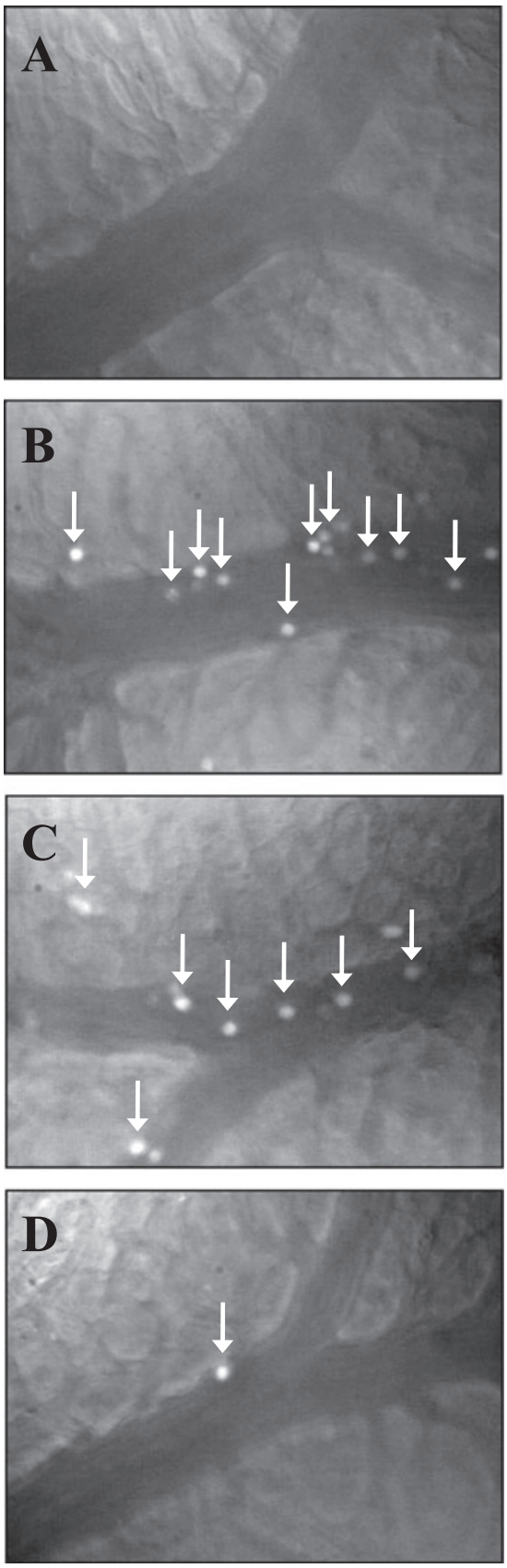

Figure 4 Representative pictures of intravital microscopy of liver tissue. Leukocytes in postsinusoidal venules and sinusoids are indicated with white arrows. Pictures were taken 12 h after ligation of the common bile duct. Mice were injected (i.p.) with either vehicle (B), simvastatin 0.02 mg·kg^{-1} (C) or 0.2 mg·kg^{-1} (D) prior to bile duct ligation (BDL). Sham animals (A) received only PBS. PBS, phosphate-buffered saline.

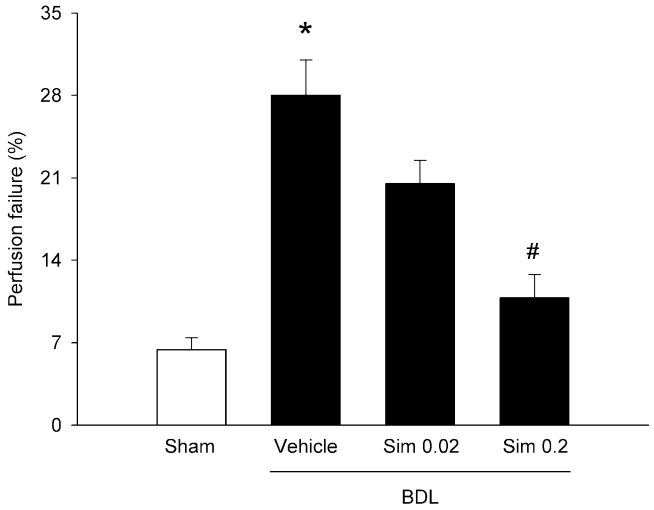

Figure 5 Sinusoidal perfusion failure 12 h after ligation of the common bile duct. Mice were treated. with vehicle and simvastatin (Sim, 0.2 and 0.02 mg·kg^{-1}, i.p.) prior to bile duct ligation (BDL). Sham animals received only PBS. Data represent means ± s.e.mean, $n = 5$. $^{*}P < 0.05$ versus Sham and $^{\#}P < 0.05$ versus vehicle+BDL. PBS, phosphate-buffered saline.

conditions with obstructed bile flow. Although the signalling mechanisms downstream of HMG-CoA reductase activity were not examined in this study, it is of interest to mention that statin-sensitive signalling molecules include Rho guanosine triphosphatases and mitogen-activated protein kinases (Okouchi *et al.*, 2003; Patel and Corbett, 2003), and most anti-inflammatory actions of statins have been ascribed to reduced prenylation of Rho guanosine triphosphatases (Laufs and Liao, 2003). In this light, it is of interest to note that we have recently observed that inhibition of Rho-kinase, which is a downstream substrate of Rho guanosine triphosphatase signalling, protects against cholestasis-induced liver injury (unpublished observation). However, the relative role of Rho-kinase signalling in this simvastatin-mediated hepatoprotection in cholestasis remains to be studied. Nonetheless, the results of the present study add cholestatic liver injury to the list of conditions that may benefit from simvastatin treatment. Other conditions known to benefit from this treatment include sepsis (Merx *et al.*, 2004), ischaemia-reperfusion (Lefer *et al.*, 1999), glomerulonephritis (Christensen *et al.*, 2006) and asthma (McKay *et al.*, 2004).

Leukocyte infiltration is a key component in immune surveillance and host-defense reactions (Springer, 1994). However, infiltration of leukocytes may cause tissue damage in certain diseases, such as ischaemia-reperfusion injury, graft rejection and endotoxaemia (Carlos and Harlan, 1994). In particular, leukocyte recruitment constitutes a rate-limiting

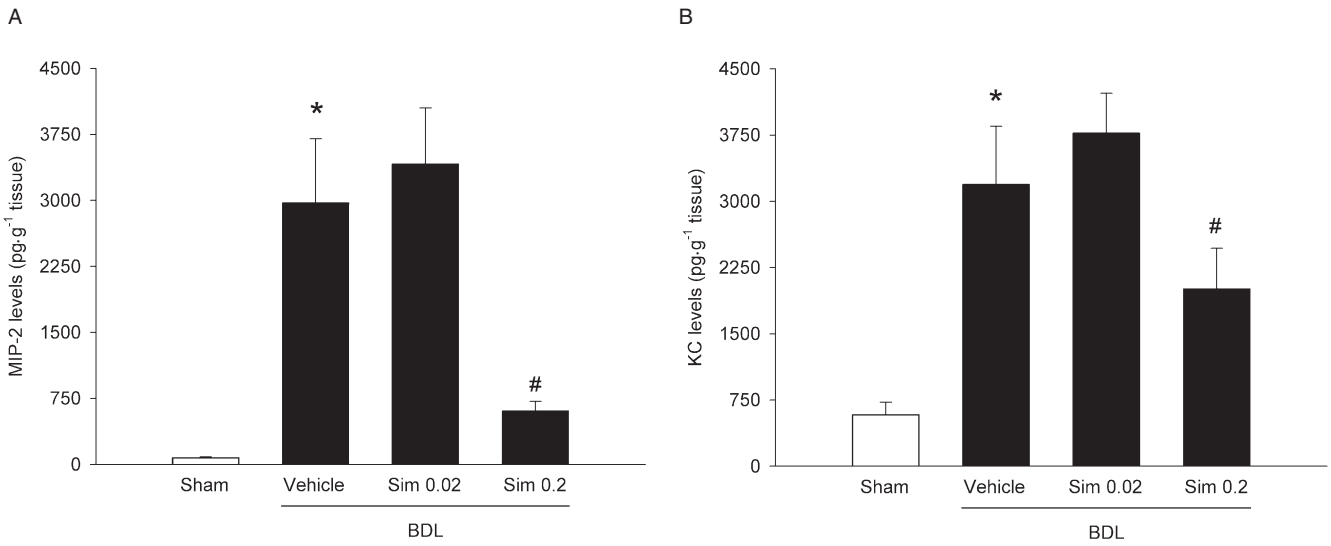

Figure 6 Hepatic levels of MIP-2 (A) and KC (B) 12 h after ligation of the common bile duct. Mice were treated with vehicle and simvastatin (Sim, 0.2 and 0.02 mg·kg^{-1}, i.p.) prior to bile duct ligation (BDL). Sham animals received only PBS. Data represent means ± s.e.mean, $n = 5$. *$P < 0.05$ versus Sham and $^{\#}P < 0.05$ versus vehicle + BDL. KC, cytokine-induced neutrophil chemoattractant; MIP-2, macrophage inflammatory protein-2; PBS, phosphate-buffered saline.

Table 1 Effect of simvastatin treatment after BDL induction

	Bilirubin (μmol·L^{-1})	*ALT (μKat·L^{-1})*	*AST (μKat·L^{-1})*	*MPO (u·g^{-1})*	*KC (pg·g^{-1})*
Sham	8.6 ± 0.1	0.24 ± 0.06	0.64 ± 0.14	0.01 ± 0.03	686 ± 106
BDL + vehicle	39.7 ± 6.0*	57.5 ± 13.2*	152.0 ± 21.3*	0.19 ± 0.08*	4009 ± 470*
BDL + Sim 0.2	48.6 ± 8.8	17.1 ± 5.1$^{\#}$	36.5 ± 10.9$^{\#}$	0.03 ± 0.05$^{\#}$	2472 ± 494$^{\#}$

Mice were treated with vehicle or simvastatin (Sim 0.2 mg·kg^{-1}) 2 h after induction of BDL. Animals not exposed to BDL served as Sham controls. Serum activities of liver enzymes were analysed in blood samples drawn from the inferior vena cava and myeloperoxidase and KC were determined in liver samples at the end of the experiments. Data are means ± s.e.mean ($n = 5$).
*$P < 0.05$ versus Sham and $^{\#}P < 0.05$ versus BDL + vehicle.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDL, bile duct ligation; KC, cytokine-induced neutrophil chemoattractant; MPO, myeloperoxidase.

step in cholestasis-induced liver injury (Gujral *et al.*, 2003; 2004). In this context, it should be mentioned that both leukocytes trapped in sinusoids and adherent in postsinusoidal venules may contribute to hepatocellular injury (Vollmar *et al.*, 1995; Chosay *et al.*, 1997; Lawson *et al.*, 1998). In the present study, we found that simvastatin decreased MPO levels, a marker of leukocyte recruitment, by 67% in the liver of cholestatic animals. In order to study leukocyte-endothelium interactions in the hepatic microcirculation in more detail, we used intravital fluorescence microscopy. By use of this technique, we observed that simvastatin significantly decreased cholestasis-induced leukocyte adhesion in both sinusoids and postsinusoidal venules. This observation adds the liver to the list of tissues, such as brain (Stanislaus *et al.*, 2001), heart (Lefer *et al.*, 1999), retina (Honjo *et al.*, 2002), colon (Sasaki *et al.*, 2003), synovium (Yamagata *et al.*, 2007) and lung (Naidu *et al.*, 2003), in which statins have been found to attenuate leukocyte infiltration. Convincing data in the literature have shown that chemokines constitute a dominating group of molecules regulating tissue accumulation of leukocytes in numerous disease models (Feng *et al.*, 1995; Schmal *et al.*, 1996; Diab *et al.*, 1999; Li *et al.*, 2004b). In the present study, we observed not only that BDL markedly increased hepatic formation of MIP-2 and KC but that pre-treatment with simvastatin also abolished cholestasis-induced CXC chemokine production in the liver. Thus, our findings suggest that simvastatin is a potent inhibitor of MIP-2 and KC formation in cholestatic liver injury. In this context, it should be noted that this study is the first to show that statins may inhibit chemokine formation in the liver. This observation extends previous findings showing that statins can attenuate production of chemokines in the heart (Shimizu *et al.*, 2003) lung (Fessler *et al.*, 2005) and brain (Nakamichi *et al.*, 2006). Thus, we predict that statins may offer a similar protection against hepatocellular damage in other models of liver disease in which CXC chemokine-dependent leukocyte recruitment constitute a significant part of the pathophysiology. Liver function is dependent on intact microvascular perfusion and oxygenation. In the present study, we observed that BDL significantly reduced blood perfusion in the hepatic sinusoids and that simvastatin treatment improved sinusoidal perfusion in cholestatic mice. As previous studies have shown that leukocyte accumulation in the hepatic microcirculation is an important factor behind perfusion failure in the liver, it is probable that the simvastatin-mediated reduction in leukocyte adhesion, at least in part, explains the improved microvascular perfusion observed in cholestatic animals treated with simvastatin observed in the present study.

In conclusion, the results of this study demonstrate that simvastatin ameliorates BDL-induced liver damage by reducing perfusion failure, CXC chemokine formation and leukocyte recruitment. Taken together, our novel findings suggest that treatment with statins may be a useful strategy for protecting against pathological inflammation in the liver associated with obstructive jaundice.

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Conflicts of interest

The authors state no conflicts of interest.

References

Carlos TM, Harlan JM (1994). Leukocyte-endothelial adhesion molecules. *Blood* **84**: 2068–2101.

Chosay JG, Essani NA, Dunn CJ, Jaeschke H (1997). Neutrophil margination and extravasation in sinusoids and venules of liver during endotoxin-induced injury. *Am J Physiol* **272**: G1195–G1200.

Christensen M, Su AW, Snyder RW, Greco A, Lipschutz JH, Madaio MP (2006). Simvastatin protection against acute immune-mediated glomerulonephritis in mice. *Kidney Int* **69**: 457–463.

Corsini A, Maggi FM, Catapano AL (1995). Pharmacology of competitive inhibitors of HMG-CoA reductase. *Pharmacol Res* **31**: 9–27.

Diab A, Abdalla H, Li HL, Shi FD, Zhu J, Höjberg B *et al*. (1999). Neutralization of macrophage inflammatory protein 2 (MIP-2) and MIP-1a attenuates neutrophil recruitment in the central nervous system during experimental bacterial meningitis. *Infect Immun* **67**: 2590–2601.

Feng L, Xia Y, Yoshimura T, Wilson CB (1995). Modulation of neutrophil influx in glomerulonephritis in the rat with anti-macrophage inflammatory protein-2 (MIP-2) antibody. *J Clin Invest* **95**: 1009–1017.

Fessler MB, Young SK, Jeyaseelan S, Lieber JG, Arndt PG, Nick JA *et al*. (2005). A role for hydroxy-methylglutaryl coenzyme a reductase in pulmonary inflammation and host defense. *Am J Respir Crit Care Med* **171**: 606–615.

Georgiev P, Jochum W, Heinrich S, Jang JH, Nocito A, Dahm F *et al*. (2008). Characterization of time-related changes after experimental bile duct ligation. *Br J Surg* **95**: 646–656.

Giusti-Paiva A, Martinez MR, Felix JV, da Rocha MJ, Carnio EC, Elias LL *et al*. (2004). Simvastatin decreases nitric oxide overproduction and reverts the impaired vascular responsiveness induced by endotoxic shock in rats. *Shock* **21**: 271–275.

Gujral JS, Farhood A, Bajt ML, Jaeschke H (2003). Neutrophils aggravate acute liver injury during obstructive cholestasis in bile duct-ligated mice. *Hepatology* **38**: 355–363.

Gujral JS, Liu J, Farhood A, Hinson JA, Jaeschke H (2004). Functional importance of ICAM-1 in the mechanism of neutrophil-induced liver injury in bile duct-ligated mice. *Am J Physiol Gastrointest Liver Physiol* **286**: G499–G507.

Honjo M, Tanihara H, Nishijima K, Kiryu J, Honda Y, Yue BY *et al*. (2002). Statin inhibits leukocyte-endothelial interaction and prevents neuronal death induced by ischemia-reperfusion injury in the rat retina. *Arch Ophthalmol* **120**: 1707–1713.

Klintman D, Schramm R, Menger MD, Thorlacius H (2002). Leukocyte recruitment in hepatic injury: selectin-mediated leukocyte rolling is a prerequisite for CD18-dependent firm adhesion. *J Hepatol* **36**: 53–59.

Klintman D, Li X, Thorlacius H (2004). Important role of P-selectin for leukocyte recruitment, hepatocellular injury, and apoptosis in endotoxemic mice. *Clin Diagn Lab Immunol* **11**: 56–62.

Laschke M, Menger MD, Wang Y, Lindell G, Jeppsson B, Thorlacius H (2007). Sepsis-associated cholestasis is critically dependent on P-selectin-dependent leukocyte recruitment in mice. *Am J Physiol Gastroenterol Hepatol Physiol* **292**: G1396–G1402.

Laufs U, Liao JK (2003). Isoprenoid metabolism and the pleiotropic effects of statins. *Curr Atheroscler Rep* **5**: 372–378.

Lawson JA, Fisher MA, Simmons CA, Farhood A, Jaeschke H (1998). Parenchymal cell apoptosis as a signal for sinusoidal sequestration and transendothelial migration of neutrophils in murine models of endotoxin and Fas-antibody-induced liver injury. *Hepatology* **28**: 761–767.

Lefer AM, Campbell B, Shin YK, Scalia R, Hayward R, Lefer DJ (1999). Simvastatin preserves the ischemic-reperfused myocardium in normocholesterolemic rat hearts. *Circulation* **100**: 178–184.

Li X, Klintman D, Weitz-Schmidt G, Schramm R, Thorlacius H (2004a). Lymphocyte function antigen-1 mediates leukocyte adhesion and subsequent liver damage in endotoxemic mice. *Br J Pharmacol* **141**: 709–716.

Li X, Klintman D, Liu Q, Sato T, Jeppsson B, Thorlacius H (2004b). Critical role of CXC chemokines in endotoxemic liver injury in mice. *J Leukoc Biol* **75**: 443–452.

McKay A, Leung BP, McInnes IB, Thomson NC, Liew FY (2004). A novel anti-inflammatory role of simvastatin in a murine model of allergic asthma. *J Immunol* **172**: 2903–2908.

Marschall HU, Wagner M, Bodin K, Zollner G, Fickert P, Gumhold J *et al*. (2006). Fxr(–/–) mice adapt to biliary obstruction by enhanced phase I detoxification and renal elimination of bile acids. *J Lipid Res* **47**: 582–592.

Merx MW, Liehn EA, Janssens U, Lütticken R, Schrader J, Hanrath P *et al*. (2004). HMG-CoA reductase inhibitor simvastatin profoundly improves survival in a murine model of sepsis. *Circulation* **109**: 2560–2565.

Naidu BV, Woolley SM, Farivar AS, Thomas R, Fraga C, Mulligan MS (2003). Simvastatin ameliorates injury in an experimental model of lung ischemia-reperfusion. *J Thorac Cardiovasc Surg* **126**: 482–489.

Nakamichi K, Saiki M, Kitani H, Kuboyama Y, Morimoto K, Takayama-Ito M *et al*. (2006). Suppressive effect of simvastatin on interferon-beta-induced expression of CC chemokine ligand 5 in microglia. *Neurosci Lett* **407**: 205–210.

Okouchi M, Okayama N, Omi H, Imaeda K, Shimizu M, Fukutomi T *et al*. (2003). Cerivastatin ameliorates high insulin-enhanced neutrophil-endothelial cell adhesion and endothelial intercellular adhesion molecule-1 expression by inhibiting mitogen-activated protein kinase activation. *J Diabetes Complications* **17**: 380–386.

Patel TR, Corbett SA (2003). Mevastatin suppresses lipopolysaccharide-induced Rac activation in the human monocyte cell line THP-1. *Surgery* **134**: 306–311.

Sasaki M, Bharwani S, Jordan P, Joh T, Manas K, Warren A *et al*. (2003). The 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor pravastatin reduces disease activity and inflammation in

dextran-sulfate induced colitis. *J Pharmacol Exp Ther* **305**: 78–85.

Schmal H, Shanley TP, Jones ML, Friedl HP, Ward PA (1996). Role for macrophage inflammatory protein-2 in lipopolysaccharide-induced lung injury in rats. *J Immunol* **156**: 1963–1972.

Shimizu K, Aikawa M, Takayama K, Libby P, Mitchell RN (2003). Direct anti-inflammatory mechanisms contribute to attenuation of experimental allograft arteriosclerosis by statins. *Circulation* **108**: 2113–2120.

Springer TA (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* **76**: 301–314.

Stanislaus R, Singh AK, Singh I (2001). Lovastatin treatment decreases mononuclear cell infiltration into the CNS of Lewis rats with experimental allergic encephalomyelitis. *J Neurosci Res* **66**: 155–162.

Stedman C, Liddle C, Coulter S, Sonoda J, Alvarez JG, Evans RM *et al*. (2006). Benefit of farnesoid X receptor inhibition in obstructive cholestasis. *Proc Natl Acad Sci USA* **103**: 11323–11328.

Terblanche M, Almog Y, Rosenson RS, Smith TS, Hackam DG (2007). Statins and sepsis: multiple modifications at multiple levels. *Lancet Infect Dis* **7**: 358–368.

Vollmar B, Glasz MD, Menger MD, Messmer K (1995). Leukocytes contribute to hepatic ischemia/reperfusion injury via intercellular adhesion molecule-1-mediated venular adherence. *Surgery* **117**: 195–200.

Weber C, Erl W, Weber KS, Weber PC (1997). HMG-CoA reductase inhibitors decrease CD11b expression and CD11b-dependent adhesion of monocytes to endothelium and reduce increased adhesiveness of monocytes isolated from patients with hypercholesterolemia. *J Am Coll Cardiol* **30**: 1212–1217.

Wong J, Johnston B, Lee SS, Bullard DC, Smith CW, Beaudet AL *et al*. (1997). A minimal role for selectins in the recruitment of leukocytes into the inflamed liver microvasculature. *J Clin Invest* **99**: 2782–2790.

Yamagata T, Kinoshita K, Nozaki Y, Sugiyama M, Ikoma S, Funauchi M (2007). Effects of pravastatin in murine collagen-induced arthritis. *Rheumatol Int* **27**: 631–639.