Hepatotoxic effects of fenofibrate in spontaneously hypertensive rats expressing human C-reactive protein

Vojtěch Škop,[1] Jaroslava Trnovská,[1] Olena Oliyarnyk,[1] Irena Marková,[1] Hana Malínská,[1] Ludmila Kazdová,[1] Václav Zídek,[2] Vladimír Landa,[2] Petr Mlejnek,[2] Miroslava Šimáková,[2] Miroslav Kůdela[3], Michal Pravenec[2], Jan Šilhavý[2]

[1]Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

[2]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

[3]Department of Forensic Medicine, Masaryk Hospital in Ústí nad Labem, Czech Republic

Correspondence Address:

Jan Šilhavý

Institute of Physiology, Czech Academy of Sciences

Vídeňská 1083

14220 Prague 4

Czech Republic

Phone: (420)241062153; Fax: (420)241062488; Email: jsilhavy@biomed.cas.cz

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SUMMARY

Dyslipidemia and inflammation play an important role in the pathogenesis of cardiovascular and liver disease. Fenofibrate has a well-known efficacy to reduce cholesterol and triglycerides. Combination with statins can ameliorate hypolipidemic and anti-inflammatory effects of fibrates. In the current study, we tested the anti-inflammatory and metabolic effects of fenofibrate alone and in combination with rosuvastatin in a model of inflammation and metabolic syndrome, using spontaneously hypertensive rats expressing the human C-reactive protein transgene (SHR-CRP transgenic rats). SHR-CRP rats treated with fenofibrate alone (100 mg/kg body weight) or in combination with rosuvastatin (20 mg/kg body weight) versus SHR-CRP untreated controls showed increased levels of proinflammatory marker IL6, increased concentrations of ALT, AST and ALP, increased oxidative stress in the liver and necrotic changes of the liver. In addition, SHR-CRP rats treated with fenofibrate, or with fenofibrate combined with rosuvastatin versus untreated controls, exhibited increased serum triglycerides and reduced HDL cholesterol, as well as reduced hepatic triglyceride, cholesterol and glycogen concentrations. These findings suggest that in the presence of high levels of human CRP, fenofibrate can induce liver damage even in combination with rosuvastatin. Accordingly, these results caution against the possible hepatotoxic effects of fenofibrate in patients with high levels of CRP.

KEY WORDS: fenofibrate; rosuvastatin; C-reactive protein; transgenic; spontaneously hypertensive rat; inflammation; hepatotoxic

INTRODUCTION

Dyslipidemia and inflammation play an important role in the pathogenesis of cardiovascular and liver disease. Fenofibrate is a lipid-lowering drug whose effects are mediated by its interactions with peroxisome proliferator-activated receptor alpha (PPARα), which regulates expression of genes that code for enzymes involved in fatty acid oxidation. Fenofibrate also increases lipoprotein lipolysis and reduces liver triglyceride production. In addition to its hypolipidemic effects, fenofibrate can reduce inflammation as evidenced by decreased levels of CRP and IL6 in both experimental studies and clinical trials (Hao et al. 2011; Coban et al. 2005; Noureldein et al. 2015; Min et al. 2012; Sun et al. 2015; Yesilbursa et al. 2005). On the other hand, the anti-inflammatory effects of fenofibrate remain controversial because in some studies no effects of fenofibrate on CRP reduction have been observed (Mulvey et al. 2012). Fenofibrate is recommended for therapy of hypertriglyceridemia and hypercholesterolemia often in combination with statins for improving its efficiency to reduce inflammation and atherogenic dyslipidemia.

Major controversy exists as to whether increased CRP contributes to the inflammation and pathogenesis of metabolic and cardiovascular disturbances or whether it is just a secondary response to inflammatory disease processes (Scirica and Morrow 2006). To test whether increased levels of human CRP *per se* can promote disturbances in glucose and lipid metabolism characteristic of metabolic syndrome, we transgenically expressed human CRP in spontaneously hypertensive rats (SHR) and demonstrated a causal role of CRP-induced inflammation in the pathogenesis of several components of metabolic syndrome in this animal model (Pravenec et al. 2011). Recently, we studied the anti-inflammatory effects of

rosuvastatin in SHR-CRP transgenic rats and in non-transgenic SHR controls, as a result of which we found that rosuvastatin treatment decreased circulating levels of inflammatory response markers IL6 and TNFα without decreasing circulating levels of human CRP. Rosuvastatin also reduced cardiac and renal inflammation and oxidative tissue damage (Šilhavý et al. 2014; Šilhavý et al. 2015). In the current study, we tested the anti-inflammatory and metabolic effects of fenofibrate alone and in combination with rosuvastatin in SHR-CRP transgenic rats.

METHODS

Animals.

Transgenic SHR (hereafter referred to as SHR-CRP) were derived by microinjections of SHR ova with a construct containing cDNA for human CRP under control of the apoE promoter in order to drive expression of the CRP transgene in the liver, where CRP is normally produced (Pravenec et al. 2011). To investigate the effects of fenofibrate (Lipanthyl, Recipharm Fontaine, Dijon, France) alone and in combination with rosuvastatin (Rosucard, Zentiva, Prague, Czech Republic) on inflammation caused by human CRP, we randomized 7-month-old transgenic SHR-CRP into 3 groups treated with placebo, fenofibrate alone (100 mg/kg body weight), and with fenofibrate in combination with rosuvastatin (20 mg/kg body weight) for 4 weeks. In each group, we studied 7 males. The rats were housed in an air-conditioned animal facility and allowed free access to their chow and water. At the end of the experiments, rats were decapitated in their postprandial state and tissues were collected for analysis. No experiments on live animals were performed. All experiments were performed in

agreement with the Animal Protection Law of the Czech Republic and were approved by the Ethics Committee of the Institute of Physiology, Czech Academy of Sciences, Prague.

Basal- and insulin-stimulated glycogen synthesis and glucose oxidation in skeletal muscle. For measurement of insulin-stimulated incorporation of glucose into glycogen and its oxidation into CO_2, diaphragmatic muscles were incubated for 2 hours in 95% O_2 + 5% CO_2 in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 0.1 μCi/ml of ^{14}C-U glucose, 5 mmol/L of unlabeled glucose, and 2.5 mg/ml of bovine serum albumin (Sigma, Fraction V, Czech Republic), with or without 250 μU/ml insulin. After the incubation, glycogen was extracted from the tissue, counted for radioactivity, and insulin-stimulated incorporation of glucose into glycogen was determined. To determine glucose oxidation, 0.2 ml of 1M hyamine hydroxide was injected into the central compartment of the incubation vial and 0.5 ml of 1M H_2SO_4 was added to the main compartment to liberate CO_2. The vials were incubated for another 45 min and the hyamine hydroxide was then quantitatively transferred to the scintillation vial containing 10 ml of toluene-based scintillation fluid for counting of radioactivity.

Glucose utilization in isolated epididymal adipose tissue. Glucose utilization in adipose tissue was determined *ex vivo* by measuring the incorporation of radioactive glucose into adipose tissue lipids. Distal parts of epididymal adipose tissue were rapidly dissected and incubated for 2 hours in Krebs-Ringer bicarbonate buffer with 5 mmol/L glucose, 0.1 μCi ^{14}C-U glucose/mL (UVVR, Prague, Czech Republic) and 2% bovine serum albumin with a gaseous phase of 95% O_2 and 5% CO_2 in the presence (250 μU/mL) or absence of insulin in

incubation media. All incubations were performed at 37°C in sealed vials in a shaking water bath. Estimation of ^{14}C-glucose incorporation into neutral lipids was performed. Briefly, adipose tissue was removed from the incubation medium, rinsed in saline, and immediately put into chloroform. The pieces of tissue were dissolved using a Teflon pestle homogenizer, methanol was added (chloroform:methanol 2:1), and lipids were extracted at 4°C overnight. The remaining tissue was removed, KH_2PO_4 was added, and a clear extract was taken for further analysis. An aliquot was evaporated, reconstituted in scintillation liquid, and the radioactivity measured by scintillation counting.

Tissue triglyceride and cholesterol measurements. In order to determine triglycerides and cholesterol in the liver, heart and gastrocnemius muscle, tissues were powdered under liquid N_2 and extracted for 16 hours in chloroform:methanol, after which 2% KH_2PO_4 was added and the solution centrifuged. The organic phase was removed and evaporated under N_2. The resulting pellet was dissolved in isopropyl alcohol, and triglyceride and cholesterol content was determined by enzymatic assay (Erba-Lachema, Brno, Czech Republic).

Liver glycogen determination. Liver tissues were hydrolyzed by boiling in 30% KOH containing oyster glycogen. After digestion, 96% ethanol was added and glycogen was allowed to precipitate at 4°C overnight. The precipitate was centrifuged, washed with ethanol, and dissolved in H_2O. Aliquots were used to measure glycogen using the glucose oxidase assay (Erba-Lachema, Brno, Czech Republic).

Biochemical analyses. Rat serum CRP and human serum CRP were measured using ELISA

kits (Alpha Diagnostics International, San Antonio, U.S.A.). Blood glucose levels were measured by the glucose oxidase assay (Erba-Lachema, Brno, Czech Republic) using tail vein blood drawn into 5% trichloracetic acid and promptly centrifuged. NEFA levels were determined using an acyl-CoA oxidase-based colorimetric kit (Roche Diagnostics GmbH, Mannheim, Germany). Serum triglyceride and cholesterol concentrations were measured using standard enzymatic methods (Erba-Lachema, Brno, Czech Republic). Serum insulin concentrations were determined using a rat insulin ELISA kit (Mercodia, Uppsala, Sweden). Serum IL6 was measured using rat ELISA kits (BioSource International, Inc., Camarillo, U.S.A.). Rat MCP-1 was measured by ELISA kits (Instant ELISA eBioscience, Austria). ALT, AST, and ALP enzyme activities were determined spectrophotometrically by routine clinical biochemistry methods using a Roche kit. HDL-cholesterol was measured after double precipitation with dextran and $MgCl_2$ by spectrophotometric methods using commercially available kits (Roche Diagnostics GmbH, Mannheim, Germany).

Parameters of oxidative stress. The activity of superoxide dismutase (SOD) was analyzed using the reaction of blocking nitrotetrazolium blue reduction and nitroformazan formation. Catalase (CAT) activity measurement was based on the ability of H_2O_2 to produce with ammonium molybdate a color complex detected spectrophotometrically. The activity of seleno-dependent glutathione peroxidase (GSH-Px) was monitored by oxidation of glutathione using Ellman's reagent (0.01M solution of 5,5'-dythiobis-2 nitrobenzoic acid). The level of reduced glutathione (GSH) was determined in the reaction of SH-groups using Ellman's reagent. The levels of reduced (GSH) and oxidized (GSSG) forms of glutathione were determined using a high-performance liquid chromatography method with fluorescent

detection according to the HPLC diagnostic kit (Chromsystems, Munich, Germany). Glutathione reductase (GR) and glutathione S-transferase (GST) activities were measured using Cayman Chemicals assay kits (Michigan, USA). Lipoperoxidation products were assessed by the levels of thiobarbituric acid reactive substances (TBARS) determined by assaying reactions with thiobarbituric acid. The levels of conjugated dienes were analyzed by extraction in media (heptane:isopropanol = 2:1) and measured spectrophotometrically in a heptane layer.

Expression of the Scd1 gene in the liver. Reverse transcription and quantitative real-time PCR analyses were performed using the TaqMan RNA-to-CT^{TM} 1-Step Kit and the TaqMan Gene Expression Assay (Life Technologies, Applied Biosystems, USA) and carried out in a Vii A^{TM} 7 Real-Time PCR System (Applied Biosystems, USA). Relative expression of the *Scd1* gene was determined after normalization against *Pgk1* (phosphoglycerate kinase 1) as an internal reference and calculated using the 2^{-Ct} method.

Histology

Pieces of tissue were fixated in 4% formaldehyde for at least 48 hours. Afterwards they were processed using standard techniques into paraffin blocks by dehydration through a series of graded ethanol baths to displace the water and infiltrate with wax. Once infiltrated, the tissues were embedded into wax blocks, which were then sectioned into slices up to 3 mm thick and dyed routinely using hematoxylin and eosin staining.

Statistical analysis. Results are expressed as means ± S.E.M. One-way ANOVA was used to

search for significant differences between the 3 groups. The Holm-Sidak method was used for all pairwise multiple comparison procedures with an overall significance level defined as $P<0.05$.

RESULTS

Effects of fenofibrate alone, and in combination with rosuvastatin, on serum levels of human CRP and rat CRP and on inflammation induced by human CRP. Table 1 shows the serum levels of human CRP and endogenous rat CRP in the different experimental groups. As shown, serum levels of human CRP were significantly reduced in SHR-CRP transgenic rats treated with fenofibrate alone and in combination with rosuvastatin when compared to untreated SHR-CRP controls. On the other hand, endogenous rat CRP levels were not affected by the treatment. Table 1 shows the effects of treatment on serum levels of IL6. Treatment with both fenofibrate alone and in combination with rosuvastatin induced significant increases in serum levels of IL6 when compared to untreated SHR-CRP controls. In addition, SHR-CRP rats treated with fenofibrate exhibited significantly higher levels of MCP-1 (Monocyte Chemoattractant Protein-1), one of the key chemokines that regulate migration and infiltration of monocytes/macrophages (Table 1).

Effects of fenofibrate alone or in combination with rosuvastatin on oxidative stress-related parameters. We assessed the effects of fenofibrate alone and in combination with rosuvastatin on human CRP-induced oxidative stress in tissues by measuring activities of antioxidative enzymes, glutathione, levels of lipoperoxidation products, conjugated dienes and TBARS

(Table 2). In the transgenic SHR-CRP strain expressing human CRP, both fenofibrate alone and in combination with rosuvastatin induced a significant increase in conjugated dienes, TBARS, and reduced GSH/GSSG ratios in the liver. On the other hand, oxidative stress did not increase in the heart and kidney cortex after treatment with fenofibrate alone and in combination with rosuvastatin. Increased oxidative stress in the liver was associated with reduced SOD, whereas CAT increased (Table 2).

Effects of fenofibrate alone and in combination with rosuvastatin on target organ damage. Table 1 shows significantly increased serum levels of ALT, AST, and ALP in SHR-CRP rats treated with fenofibrate alone and in combination with rosuvastatin when compared to untreated SHR-CRP controls. SHR-CRP rats treated with fenofibrate alone and in combination with rosuvastatin exhibited similar changes in liver tissue (Figures 1B and 1C), which ranged from dystrophy, presented by hydropic changes of hepatocytes with granulation and vacuolization of the cytoplasm, to signs of centrilobular reparation, to focal necrosis of hepatocytes with leukocyte infiltration. In contrast, microscopic examination of the livers of untreated SHR-CRP rats revealed only mild dystrophic changes with centrilobular reparation (Figure 1A). In kidneys, chronic changes including diffuse hyaline deposition in glomeruli were found, however, no inflammatory or necrotic changes were present (data not shown).

Metabolic effects of fenofibrate alone and in combination with rosuvastatin. As shown in Table 1, treatment of SHR-CRP rats with fenofibrate alone and in combination with rosuvastatin when compared to untreated controls was associated with increased serum triglyceride and reduced HDL cholesterol levels, with reduced hepatic triglyceride and

glycogen concentrations but increased liver weight, increased basal and insulin stimulated incorporation of glucose in epididymal fat triglycerides, and higher glucose oxidation in skeletal muscle.

Expression of Scd1 (stearoyl-CoA desaturase) in the liver. Treatment with fenofibrate alone and in combination with rosuvastatin was associated with a more than 100-fold increase in hepatic expression of the *Scd1* gene (Figure 2).

DISCUSSION

In the current study, we found that treatment of SHR-CRP rats with fenofibrate alone and in combination with rosuvastatin induced liver damage, including increased serum levels of ALT, AST, and ALP, oxidative stress (increased levels of lipoperoxidation products, reduced GSH/GSSG ratio, and reduced activity of SOD) and dystrophic, inflammatory, and necrotic changes revealed by histology. In addition, the treatment was associated with increased systemic inflammation, as evidenced by high levels of serum IL6 and MCP-1 in SHR-CRP rats treated with fenofibrate alone and in combination with rosuvastatin. On the other hand, treatment with fenofibrate in combination with rosuvastatin was associated with significant reduction of transgenic human CRP, whereas treatment with rosuvastatin alone had no effects on transgenic human CRP (Šilhavý et al. 2014). Since the human CRP transgene is under the control of the ApoE (Apolipoprotein E) promoter, it is possible that fenofibrate can reduce the expression of the transgene by interacting with the ApoE promoter, similar to its negative effects on apolipoprotein A-I expression (Vu-Dac et al. 1994). The lack of beneficial effects

of reduced human CRP levels might be explained by the fact that this decrease was not sufficient to ameliorate the adverse effects of transgenic human CRP. Mild, transiently increased levels of serum aminotransferases were reported in up to 20% of patients treated with fenofibrate (Kobayashi et al. 2009; Geng et al. 2013; Guo et al. 2012). However, increased levels of aminotransferases in patients treated with fenofibrate are usually asymptomatic and transient. On the other hand, there have also been multiple reports that patients treated with fenofibrate develop liver injury (Livertox 2015).

The treatment with fenofibrate alone and in combination with rosuvastatin was associated with a more than 100-fold increase in hepatic *Scd1* expression. *Scd1* gene codes for stearoyl-CoA desaturase that catalyzes production of monounsaturated fatty acids, which serve as substrates for the synthesis of triglycerides, cholesteryl esters, and phospholipids. *Scd1* also plays an important role in inflammation (Liu et al 2011). Its expression is under the control of transcription factors SREBP and LXR, which are regulated by PPARα (Hebbachi et al. 2008). It can be therefore expected that the effect of fenofibrate on *ScdI* is mediated by PPARα activation. It has been demonstrated in a rat model of non-alcoholic steatohepatitis (NASH) (rats fed a methionine and choline-deficient diet) that pharmacological inhibition of *Scd1* is associated with reduced hepatic steatosis, lower ALT and AST levels, and amelioration of hepatocellular degeneration and inflammation (Kurikawa et al. 2013). These findings are in agreement with the results of the current study, where increased expression of *Scd1* was associated with hepatotoxic effects. On the other hand, more than a 200-fold increase in hepatic *Scd1* expression was observed in Wistar rats fed a standard diet and treated with fenofibrate, while no increase in *Scd1* expression was observed in untreated controls

(Yamazaki et al. 2012). No liver damage was reported in Wistar rats treated with fenofibrate, which suggests that fenofibrate alone is not hepatotoxic in Wistar rats and that its adverse effects on the liver are likely to be caused in combination with additional harmful factors, such as high fat and high fructose diets and inflammation. Thus, it is possible that in our study the combined adverse effects of high levels of human CRP and fenofibrate resulted in severe hepatotoxicity.

SHR-CRP rats treated with fenofibrate alone and in combination with rosuvastatin showed significantly increased oxidative stress in the liver, with significantly higher levels of conjugated dienes and TBARS and lower GSH/GSSG ratios compared to untreated controls. On the other hand, no significant increase of lipoperoxidation products was observed in the heart and kidney cortex after treatment. In our previous studies (Šilhavý et al. 2014), SHR-CRP rats treated with rosuvastatin alone exhibited significantly reduced levels of both conjugated dienes and TBARS and lower levels of serum IL6 when compared to untreated rats. Despite these observations, combined treatment with fenofibrate and rosuvastatin in SHR-CRP rats resulted in similar adverse effects to treatment with fenofibrate alone. Although the mechanisms of the hepatotoxic effects of fenofibrate are not known, we suggest that they are, at least in part, caused by tissue damage due to oxidative stress. Fenofibrate, through the activation of PPARα, causes the proliferation of peroxisomes and an increased number of peroxisomal enzymes, including acyl-CoA oxidase (ACO). This results in massive generation of H_2O_2 (Nemali et al. 1989; Yeldandi et al. 2000; Peters et al. 2005). The increased quantity of H_2O_2 is not sufficiently eliminated by catalase, even though its activity is also increased by fenofibrate. Increased H_2O_2 can react with cellular components and cause

oxidative stress and, consequently, cell and tissue damage.

The treatment of SHR-CRP rats with fenofibrate was associated with reduced concentrations of liver triglycerides, cholesterol, and glycogen along with increased serum triglyceride and reduced HDL cholesterol levels when compared to untreated SHR-CRP controls. Inflammation is associated with increased plasma triglyceride levels due to increased lipolysis in adipose tissue and suppressed fatty acid uptake and oxidation in skeletal muscle, shifting its metabolism from fatty acids to glucose as a preferred fuel substrate (Khovidhunkit et al. 2004). In our previous studies in SHR-CRP transgenic rats treated with rosuvastatin alone versus SHR-CRP untreated controls, we observed significantly reduced serum triglycerides. In contrast, cholesterol levels were unchanged and hepatic triglycerides and oxidative stress were significantly reduced (Šilhavý et al. 2014). These beneficial effects of rosuvastatin were not observed in the current study, where treatment of SHR-CRP rats with combination of fenofibrate and rosuvastatin was associated with increased serum triglycerides and liver oxidative stress.

In conclusion, the results of the current study strongly suggest that, in the presence of high levels of human CRP, fenofibrate can induce liver damage even in combination with rosuvastatin, which has been previously shown to protect against the adverse effects of human CRP in this animal model. Accordingly, these findings caution against the possible hepatotoxic effects of fenofibrate in patients with high levels of CRP.

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Table 1 Biochemical and metabolic parameters in SHR-CRP transgenic rats treated with placebo, fenofibrate alone, and in combination with rosuvastatin.

Trait	SHR-CRP placebo	SHR-CRP fenofibrate	SHR-CRP fenofibrate + rosuvastatin
Body weight (g)	336±6	349±8	324±11
Relative weight of epididymal fat (g/100 g body weight)	0.736±0.048	0.798±0.029	0.807±0.049
Relative liver weight (g/100 g body weight)	3.56±0.06	5.93±0.07[a]	6.17±0.29[b]
Serum glucose (mmol/L)	7.6±0.1	7.0±0.2	7.3±0.2
Serum insulin (nmol/L)	0.450±0.038	0.337±0.052	0.387±0.041
Serum triglycerides (mmol/L)	0.58±0.06	0.84±0.08[a]	0.94±0.08[b]
Serum HDL (mmol/L)	1.00±0.04	0.70±0.09[a]	0.72±0.03[b]
Serum cholesterol (mmol/L)	1.31±0.06	1.26±0.11	1.05±0.04[b]
Serum IL6 (pg/ml)	6.5±1.2	50.3±10.4[a]	36.4±9.7[b]
Serum MCP-1 (ng/ml)	12.2±0.7	17.7±2.6[a]	12.6±1.6
Rat CRP (μg/ml)	97±9	114±4	121±10
Human CRP (μg/ml)	872±81	456±30[a]	495±53[b]
Serum ALT	2.8±0.2	15.8±1.4[a]	9.6±1.7[b]
Serum AST	6.0±0.3	18.9±2.2[a]	11.5±1.4[b]
Serum ALP	3.2±0.1	11.3±0.5[a]	9.7±0.3[b]
Liver triglycerides (μmol/g)	6.3±0.3	3.6±0.2[a]	3.9±0.3[b]
Liver glycogen (mmol/g)	2.47±0.12	0.47±0.05[a]	0.47±0.04[b]
Basal lipogenesis (nmol gl./mg/2 h)	440±60	1095±112[a]	1676±183[bc]
Stimulated lipogenesis (nmol gl./mg/2 h)	1037±78	2023±189[a]	2621±184[bc]
Basal glycogenesis (nmol gl./mg/2 h)	782±58	655±90	677±65
Stimulated glycogenesis (nmol gl./mg/2 h)	1326±95	1235±118	1084±151
Glucose oxidation in muscle (nmol gl./mg/2 h)	160±12	206±14[a]	240±16[b]

One-way ANOVA results. The Holm-Sidak method was used for all pairwise multiple comparison procedures. [a] denotes $P<0.05$ for comparison between placebo vs. fenofibrate; [b] denotes $P<0.05$ for comparison between placebo vs. fenofibrate + rosuvastatin; [c] denotes $P<0.05$ for comparison between fenofibrate vs. fenofibrate + rosuvastatin.

Table 2 Parameters of oxidative stress in tissues isolated from SHR-CRP transgenic rats treated with placebo, fenofibrate alone, and in combination with rosuvastatin.

Trait	SHR-CRP placebo	SHR-CRP fenofibrate	SHR-CRP fenofibrate + rosuvastatin
	Liver		
SOD (U/mg)	0.136±0.011	0.103±0.004[a]	0.104±0.003[b]
GSH-Px (μM NADPH/min/mg)	301±32	263±22	269±12
GR (μM NADPH/min/mg)	123±9	132±8	168±8[bc]
GST (nM CDNB/min/mg)	130±6	157±9	174±8[c]
CAT (mM H_2O_2/min/mg)	1283±56	1666±124	1839±136[b]
GSH (μmol/mg prot.)	75±5	59±5[a]	59±2[b]
GSSG (μmol/mg prot.)	3.2±0.4	4.2±0.5	4.1±0.5
GSH/GSSG	26.2±2.7	15.3±2.4[a]	15.6±1.9[b]
Conjugated dienes (nM/mg)	31.3±1.8	45.3±4.1[a]	47.9±2.2[b]
TBARS (nM/mg)	1.332±0.070	2.018±0.132[a]	1.911±0.147[b]
	Heart		
SOD (U/mg)	0.063±0.005	0.074±0.004	0.075±0.007
GSH-Px (μM NADPH/min/mg)	118±7	151±17	183±18[b]
GR (μM NADPH/min/mg)	46±3	41±5	49±4
GST (nM CDNB/min/mg)	28±2	27±2	29±3
CAT (mM H_2O_2/min/mg)	603±32	591±30	612±44
Conjugated dienes (nM/mg)	15.6±1.3	14.5±1.0	12.8±1.5
TBARS (nM/mg)	0.724±0.071	0.766±0.056	0.547±0.043[c]
	Kidney cortex		
SOD (U/mg)	0.067±0.007	0.074±0.004	0.075±0.007
GSH-Px (μM NADPH/min/mg)	63±7	44±4	47±4
GR (μM NADPH/min/mg)	53±4	50±5	47±2
GST (nM CDNB/min/mg)	57±3	74±6[a]	72±4[b]
CAT (mM H_2O_2/min/mg)	646±31	796±40[a]	576±34[c]
Conjugated dienes (nM/mg)	20.7±1.0	19.1±2.1	21.9±2.1
TBARS (nM/mg)	0.638±0.042	0.547±0.046	0.669±0.037

One-way ANOVA results. The Holm-Sidak method was used for all pairwise multiple comparison procedures. [a] denotes $P<0.05$ for comparison between placebo vs. fenofibrate; [b] denotes $P<0.05$ for comparison between placebo vs. fenofibrate + rosuvastatin; [c] denotes $P<0.05$ for comparison between fenofibrate vs. fenofibrate + rosuvastatin.

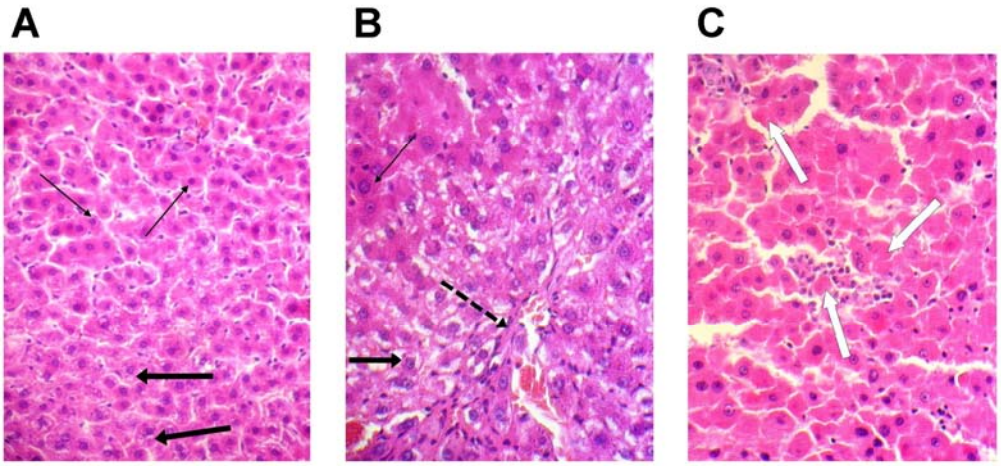

Figure 1. Histological examination of livers of SHR-CRP transgenic rats treated with placebo (A), fenofibrate alone, (B) and in combination with rosuvastatin (C). Thin arrow: normal hepatocyte; thick arrow: dystrophic hepatocyte; dashed arrow: site of reparation; empty arrows: necrotic hepatocytes with leukocyte infiltration.

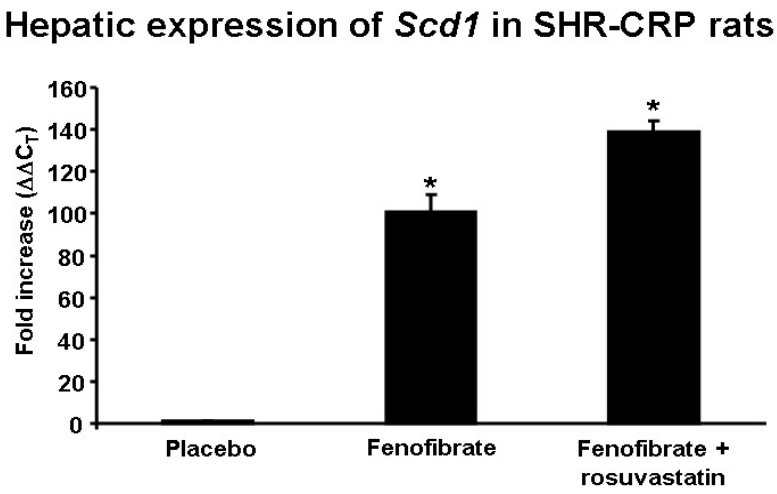

Figure 2. Hepatic expression of the *Scd1* gene in SHR-CRP transgenic rats treated with fenofibrate alone, fenofibrate in combination with rosuvastatin, and with placebo. * denotes $P<0.0001$.