Combination Therapy with Pemafibrate (K-877) and Pitavastatin Improves Vascular Endothelial Dysfunction in Dahl/Salt-sensitive Rats Fed a High-salt and High-fat Diet

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Abstract

Background

Statins suppress the progression of atherosclerosis by reducing low-density lipoprotein (LDL) cholesterol levels. Pemafibrate (K-877), a novel selective peroxisome proliferator-activated receptor α modulator, is expected to reduce residual risk factors including high triglycerides (TGs) and low high-density lipoprotein (HDL) cholesterol during statin treatment. However, it is not known if statin therapy with add-on pemafibrate improves the progression of atherosclerosis. The aim of this study was to assess the effect of combination therapy with pitavastatin and pemafibrate on lipid profiles and endothelial dysfunction in hypertension and hyperlipidemia model rats.

Methods

Seven-week-old male Dahl salt-sensitive (DS) rats were divided into the following five treatment groups (normal diet (ND) plus vehicle, high-salt and high-fat diet (HD) plus vehicle, HD plus pitavastatin (0.3 mg/kg), HD plus pemafibrate (K-877) (0.5 mg/kg), and HD plus combination of pitavastatin and pemafibrate) and treated for 12 weeks. At 19 weeks, endothelium-dependent relaxation of the thoracic aorta in response to acetylcholine was evaluated.

Results

After feeding for 12 weeks, systolic blood pressure and plasma levels of total cholesterol were significantly higher in the HD-vehicle group compared with the ND-vehicle group. Combination therapy with pitavastatin and pemafibrate significantly reduced systolic blood pressure, TG levels, including total, chylomicron (CM), very LDL (VLDL), HDL-TG, and cholesterol levels, including total, CM, VLDL, and LDL-cholesterol, compared with vehicle treatment. Acetylcholine caused concentration-dependent relaxation of thoracic aorta rings that were pre-contracted with phenylephrine in all rats. Relaxation rates in the HD-vehicle group were significantly lower compared with the ND-vehicle group. Relaxation rates in the HD-combination of pitavastatin and pemafibrate group significantly increased compared with the HD-vehicle group, although neither medication alone ameliorated relaxation rates significantly. Western blotting experiments showed increased phosphorylated endothelial nitric oxide synthase protein expression in aortas from rats in the HD-combination group.
compared with the HD-vehicle group. However, the expression levels did not respond significantly to either medication alone.

Conclusions
Combination therapy with pitavastatin and pefamibrate improved lipid profiles and endothelial dysfunction in hypertension and hyperlipidemia model rats. Pefamibrate as an add-on strategy to statins may be useful for preventing atherosclerosis progression.

Background
Many clinical trials and meta-analyses have revealed that treatment with statins, which are 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, targets a reduction in low-density lipoprotein cholesterol (LDL-C) and thereby decreases the risk of coronary heart disease (CHD) and all-cause mortality [1]. However, many CHD cases are not prevented and the residual risk factors including high triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) levels remains unclear [2].

Fasting and non-fasting (or postprandial) hypertriglycerideremia is a risk factor for CHD [3–5]. Several mechanisms of atherogenesis in hypertriglycerideremia are proposed. Among them, postprandial or non-fasting hypertriglycerideremia is involved in the production of proinflammatory cytokines, recruitment of neutrophils, and generation of oxidative stress, resulting in endothelial dysfunction [6–9]. Endothelial dysfunction is an initial process of atherogenesis, and it contributes to the pathogenesis of CHD. Among TG-rich lipoproteins, remnant lipoproteins depress the activity of endothelial nitric oxide synthase (eNOS) in endothelial cells and decrease nitric oxide (NO) released from the endothelium [10, 11].

We and other investigators also reported that a postprandial increase of serum TG and remnant-like particles-cholesterol (RLP-C) causes endothelial dysfunction assessed by brachial artery flow-mediated dilatation (FMD) [5, 6, 12, 13]. FMD has been shown to be impaired in patients with traditional coronary risk factors, including hypertension, dyslipidemia, diabetes mellitus, and smoking, and it has been considered to be a cause of atherosclerosis [14]. Statins improve endothelial function as assessed by FMD [15, 16]. In addition, hypertriglycerideremia is independently associated with
endothelial dysfunction as assessed by FMD in patients with CHD during statin therapy [17].

Previously, a clinical trial for combination therapy with a statin plus a fibrate to reduce the residual risk was conducted in patients with type 2 diabetes mellitus. The ACCORD study showed that combination therapy with simvastatin plus fenofibrate did not reduce cardiovascular outcomes and mortality compared with simvastatin alone [18]. However, in a preplanned subgroup analysis, there was a trend benefit of fenofibrate in patients with a high TG level (≥ 204 mg/dL) or a low HDL-C level (≤ 34 mg/dL) [2]. Additionally, fibrate treatment during the trial period was associated with a legacy effect of improved survival over a post-trial follow-up [19]. These findings suggest that re-evaluation of TG-lowering therapy as an add-on strategy to statins is needed.

Fibrates activate a transcription factor that belongs to the nuclear receptor superfamily, peroxisome proliferator-activated receptor α (PPARα), and controls lipid metabolism. Recently, pemafibrate (K-877), a novel selective PPARα modulator (SPPARMα), has been developed [20], which has even more potent and selective activity against PPARα. Pemafibrate robustly decreases serum TG levels in fasting and non-fasting or postprandial states and increases serum HDL-C levels [21, 22]. Moreover, pemafibrate was superior to fenofibrate in terms of serum TG-lowering effect and hepatic and renal safety [21]. The ongoing PROMINENT trial is ongoing in patients with type 2 diabetes mellitus, elevated TG, and low levels of HDL-C to determine whether treatment with pemafibrate safely reduces residual cardiovascular risk. Therefore, we hypothesized that combination therapy with a statin plus pemafibrate could notably improve endothelial dysfunction in hyperlipidemia under postprandial or non-fasting condition. The aim of this study was to assess the effect of combination therapy with pitavastatin and pemafibrate on lipid profiles and endothelial dysfunction in Dahl salt-sensitive (DS) rats fed a high-salt and high-fat diet, which showed hypertension and hyperlipidemia in the non-fasting state.

Methods
Protocols for animal experiments
Seven-week-old male Dahl salt-sensitive (DS) rats (n = 44) (Japan SLC, Shizuoka, Japan) were fed a normal diet (ND; 0.3% NaCl and 4.5% fat) (CE-2, CLEA Japan, Inc., Tokyo, Japan) or a high-salt and
high-fat diet (HD; 8% NaCl and 29.4% fat) (CLEA Japan), as previously described [23], and they were treated with a vehicle, pitavastatin (0.3 mg/kg) (Kowa Co., Ltd., Tokyo, Japan), pemafibrate (K-877) (0.5 mg/kg) (Kowa Co., Ltd.) or a combination of pitavastatin (0.3 mg/kg) and pemafibrate (K-877) (0.5 mg/kg) (Fig. 1) for a period of 12 weeks (Fig. 1). Rats were divided into the following five groups: (1) ND-vehicle group (n = 5) fed an ND and treated with vehicle; (2) HD-vehicle group (n = 9) fed an HD and treated with vehicle; (3) HD-pitavastatin group (n = 10) fed an HD and treated with pitavastatin; (4) HD-pemafibrate group (n = 10) fed an HD and treated with pemafibrate; and (5) HD-combination group (n = 10) fed an HD and treated with combination of pitavastatin and pemafibrate. All experimental protocols were approved by and conducted in accordance with the recommendations of the Okayama University Animal Care and Use Committee (permit number OKU-2019349).

**Blood pressure and pulse rate measurement**

Systolic blood pressure and pulse rate were measured at 12 and 19 weeks using a tail-cuff plethysmography (MK-2000 Muromachi, Tokyo, Japan or BP-2000, Visitech Systems, Inc., Apex, NC, USA). An average of three measurements was used.

**Blood collection and measurements**

At 19 weeks, rats were anesthetized with isoflurane. Whole blood was collected from abdominal aorta into a chilled tube. After centrifugation at 3000 x g for 10 minutes at 4 °C, plasma was collected and stored at −80 °C. Plasma total bilirubin was measured using the vanadate oxidase method. Plasma glucose was measured using the hexokinase/glucose-6-phosphate dehydrogenase method. Plasma cholesterol and TG content in lipoprotein fractions including chylomicron (CM), very low-density lipoprotein (VLDL), LDL, and HDL were analyzed using high-performance liquid chromatography by Skylight Biotech (Akita, Japan), as described [24, 25].

**Vascular relaxation studies**

Endothelium-dependent relaxation in response to acetylcholine was evaluated. At 19 weeks, rats were anesthetized with isoflurane. The thoracic aorta was rapidly removed, gently cleaned taking care not to damage the endothelium, and it was cut into 3-mm rings. The rings were then cut open. Open aortic rings were placed in a 10-mL organ bath containing Krebs – Henseleit solution (KHS; in mmol/L: 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 11.1 glucose). One end of the open
ring was connected to a tissue holder and the other end was connected to a force displacement transducer (AD-611J, Nihon Kohden, Tokyo, Japan). The bathing solution was gassed with 95% O₂ and 5% CO₂ at 37 °C (pH 7.4). The tissue was equilibrated for 60 min under a resting tension of 1 g. During this time, Krebs – Henseleit solution was replaced every 15 min with fresh solution. The tissues were pre-contracted with phenylephrine (0.3 µmol/L). Tissues were then re-washed and pre-contracted with phenylephrine (0.3 µmol/L). After the phenylephrine-induced contraction had reached a plateau, the concentration-response relationships for acetylcholine (1–10000 nmol/L) were obtained by adding acetylcholine to the bath in a cumulative manner. Finally, papaverine (100 µmol/L) relaxation responses were obtained. The relaxation responses obtained were expressed as a percentage of the maximal relaxation that was evoked by papaverine (100 µmol/L).

Western blot analysis
Cardiac tissue protein samples were prepared using a BEAD crusher (µT-12, Taitec, Koshigaya, Japan). Tissue lysates were extracted in radioimmunoprecipitation (RIPA) buffer with 2 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L sodium orthovanadate, and 10 mmol/L sodium fluoride (sc-24948, Santa Cruz, Dallas, Texas, USA). Rabbit anti-eNOS antibody (#32027, Cell Signaling, Danvers, MA, USA), rabbit anti-phospho-eNOS (Ser1177) antibody (#9571, Cell Signaling), and mouse anti-beta actin antibody (ab6276, Abcam, Cambridge, UK) were used. The second antibody was horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG antibody (NA934 and NA931, GE Healthcare Bio-Sciences, Buckinghamshire, England). Positive signals were detected using a chemiluminescence system (ECL plus, GE Healthcare Bio-Sciences).

Statistical analysis
Statistical analysis was performed using SPSS version 24 (IBM, New York, USA). All results are expressed as the mean ± standard deviation (SD). For comparison between different treatment groups, statistical analysis was performed using a one-way analysis of variance (ANOVA) with a Bonferroni post-hoc test. Vascular relaxation studies were analyzed using a mixed effect model with a Bonferroni post-hoc test. P-values < 0.05 were considered to be significant.

Results
Effects of pitavastatin, pemafibrate, or a combination of pitavastatin and pemafibrate on blood
pressure and heart rate
After feeding for 12 weeks, systolic blood pressure was significantly higher in the HD-vehicle group compared with the ND-vehicle group (HD-vehicle group versus ND-vehicle group, P < 0.001; Table 1). After treatment for 12 weeks, systolic blood pressure was significantly lower in the HD-combination group compared with the HD-vehicle group, the HD-pitavastatin group, and the HD-pemafibrate group (HD-combination group versus HD-vehicle group, P < 0.005; versus HD-pitavastatin group, P < 0.001; and versus HD-pemafibrate group, P < 0.01; Table 1). We observed no difference in heart rate among the five groups at 12 weeks post-feeding and treatment.

Effects of pitavastatin, pemafibrate, or a combination of pitavastatin and pemafibrate on blood measurements
After feeding for 12 weeks, plasma levels of total cholesterol were significantly higher in the HD-vehicle group compared with the ND-vehicle group (P < 0.001; Table 1 and Fig. 2A). For lipoprotein fractions, plasma LDL-cholesterol and HDL-cholesterol levels were significantly higher in the HD-vehicle group compared with the ND-vehicle group (LDL, HD-vehicle group versus ND-vehicle group, P < 0.001; and HDL, HD-vehicle group versus ND-vehicle group P < 0.001; Fig. 2C, D and E). Treatment with pemafibrate and a combination of pitavastatin and pemafibrate significantly reduced plasma total cholesterol levels compared with vehicle treatment (HD-vehicle group versus HD-pemafibrate group, P < 0.005; and HD-vehicle group versus HD-combination group, P < 0.001; Table 1 and Fig. 2A). For lipoprotein fractions, plasma CM-cholesterol, VLDL-cholesterol, and LDL-cholesterol levels were significantly lower in the HD-combination group compared with the HD-vehicle group (CM, HD-combination group versus HD-vehicle group, P < 0.05; VLDL, HD-combination group versus HD-vehicle group, P < 0.001; and LDL, HD-combination group versus HD-vehicle group, P < 0.001; Fig. 2B, C and D). Plasma VLDL-cholesterol and LDL-cholesterol levels were also significantly lower in the HD-pemafibrate group compared with the HD-vehicle group (VLDL, HD-pemafibrate group versus HD-vehicle group, P < 0.001; and LDL, HD-pemafibrate group versus HD-vehicle group, P < 0.001; Fig. 2C and D).
There were no significant differences in plasma total TG levels between the ND-vehicle group and the HD-vehicle group at 12 weeks post-feeding (Table 1 and Fig. 3A).
Treatment with pemafibrate and a combination of pitavastatin and pemafibrate significantly reduced plasma total TG levels compared with vehicle treatment (HD-vehicle group versus HD-pemafibrate group, P < 0.05 and HD-vehicle group versus HD-combination group, P < 0.01; Table 1 and Fig. 3A). For lipoprotein fractions, plasma CM-TG, VLDL-TG, and HDL-TG levels were significantly lower in the HD-pemafibrate group and the HD-combination group compared with the HD-vehicle group (CM, HD-pemafibrate group versus HD-vehicle group, P < 0.05; HD-combination group versus HD-vehicle group, P < 0.01; VLDL, HD-pemafibrate group versus HD-vehicle group, P < 0.01; HD-combination group versus HD-vehicle group, P < 0.005; and HD-combination group versus HD-vehicle group, P < 0.01; Fig. 3B, C and E).

There were no significant differences in plasma total bilirubin and plasma glucose levels among the five groups at 12 weeks post-feeding and treatment.

Effects of pitavastatin, pemafibrate, or a combination of pitavastatin and pemafibrate on endothelium-dependent vascular relaxation in response to acetylcholine

Acetylcholine (1 – 10000 nmol/L) caused concentration-dependent relaxation in thoracic aorta rings that were pre-contracted by phenylephrine (0.3 µmol/L) in all five groups (Fig. 4). Relaxation rates in the HD-vehicle group were significantly lower compared with the ND-vehicle group (HD-vehicle group versus ND-vehicle group, P < 0.001). Relaxation rates in the HD-combination of pitavastatin and pemafibrate group significantly increased compared with those in the HD-vehicle group (HD-combination group versus HD-vehicle group, P < 0.05), although neither medication alone ameliorated relaxation rates significantly.

Effects of pitavastatin, pemafibrate, or a combination of pitavastatin and pemafibrate on total and phospho-eNOS expression of aorta

Western blotting experiments revealed that there was no significant difference in total eNOS protein expression between aortas from rats of HD-vehicle, HD-pitavastatin, HD-pemafibrate, and HD-combination groups (Fig. 5A and B). Increased expression of phosphorylated eNOS (phospho-eNOS) on Ser1177 proteins was observed in aortas from rats in the HD-combination group compared with those from rats in the HD-vehicle group (HD-combination group versus HD-vehicle group, P < 0.05; Fig. 5A and B). However, the expression levels did not respond significantly to either medication
Discussion

The major new finding of this work is that combination therapy with pitavastatin and pemafibrate can improve endothelial dysfunction in hypertension and hyperlipidemia model rats, although neither medication alone ameliorated endothelial function significantly.

Additionally, combination therapy significantly reduced systolic blood pressure, TG levels including total, CM, VLDL, and HDL-TG, and cholesterol levels, including total, CM, VLDL, LDL-cholesterol, compared with vehicle treatment and increased expression of phosphorylated eNOS proteins in aortas. These results are considered to be a possible cause of the beneficial effects of combination therapy on endothelial function.

A double-blind, placebo-controlled, phase 2 clinical trial revealed that pemafibrate decreased TG, VLDL-cholesterol, CM-cholesterol, remnant lipoprotein cholesterol, apolipoprotein B, and apolipoprotein C-III levels and increased levels of HDL-cholesterol levels in dyslipidemic patients with high TG and low HDL-cholesterol [26]. This study also showed that pemafibrate decreased TG, including total, CM, VLDL, and HDL-TG, and cholesterol, including total, VLDL, and LDL, in dyslipidemic rats with high TG and high cholesterol levels. Therefore, pemafibrate significantly reduced TG-rich protein levels based on the clinical settings. Takei et al. reported that pemafibrate (K-877) is a potential PPARα-modulating drug to treat hyperlipidemia that works well in both the liver and small intestine of LDL receptor knockout (Ldlr−/−) mice [27]. Our study also revealed that pemafibrate improved VLDL-TG and CM-TG, and thus, pemafibrate had beneficial effects on the liver and small intestine in our model. Because the model showed high HDL-cholesterol levels rather than low HDL-cholesterol levels, pemafibrate did not increase the HDL-cholesterol levels.

HMG-CoA reductase inhibitors (statins) did not lower plasma cholesterol in rats and only at high doses (> 100-fold that required to inhibit cholesterol synthesis) did cholesterol lowering occur in chow-fed rats with statins [28]. In accordance with previous studies, pitavastatin did not reduce cholesterol levels significantly in this study with a rat model. However, combination therapy with pitavastatin and pemafibrate significantly decreased TG levels, including total, CM, VLDL, and HDL-TG, and cholesterol
levels, including total, CM, VLDL, and LDL, in dyslipidemic rats with high TG and high cholesterol. A double-blind, placebo-controlled clinical trial also revealed that pemafibrate add-on therapy in combination with pitavastatin treatment showed a robust reduction of TG in patients with dyslipidemia. These results support the favorable effects of combination therapy on lipid profiles in dyslipidemic conditions.

Combination therapy with pitavastatin and pemafibrate significantly improved systolic blood pressure compared with vehicle treatment in salt-sensitive hypertensive rats fed a high-salt and high-fat diet. Gilbert et al. also reported that the PPARα agonist fenofibrate lowers blood pressure in salt-sensitive hypertensive patients [29]. The precise mechanism of this phenomenon remains unclear, and further studies are needed to clarify this point.

Increased expression of phospho-eNOS on Ser1177 proteins was observed in aortas from rats in the HD-combination group compared with those from rats from the HD-vehicle group. Because Ser1177 phosphorylation has been shown to increase NO production from eNOS [30], this reaction might have contributed to the improvement of endothelial function using combination therapy.

Conclusions
Combination therapy with pitavastatin and pemafibrate improved lipid profiles including TG and cholesterol levels and ameliorated endothelial dysfunction. There was also an increase in the expression level of phospho-eNOS on Ser1177 protein in hypertension and hyperlipidemia model rats. Pemafibrate as an add-on strategy to statins may be useful for preventing atherosclerosis progression.

Abbreviations
HMG-CoA, 3-hydroxy-methylglutaryl coenzyme A; LDL-C, low-density lipoprotein cholesterol; CHD, coronary heart disease; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; RLP-C, remnant-like particles-cholesterol; FMD, flow-mediated dilatation; PPARα, peroxisome proliferator-activated receptor α; SPPARMα, selective PPARα modulator; DS, Dahl salt-sensitive; ND, normal diet; HD, high-fat diet; CM, chylomicron; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; KHS, Krebs-
Henseleit solution; RIPA, radioimmunoprecipitation; phospho-eNOS, phosphorylated eNOS; Ldlr\(^{-/-}\), LDL receptor knockout

**Declarations**

**Ethics approval and consent to participate**

All experimental protocols were approved by and conducted in accordance with the recommendations of the Okayama University Animal Care and Use Committee (permit number OKU-2019349).

**Consent for publication**

Not applicable

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Competing interests**

Nakamura K, Miyoshi T, and Ito H received speaker honoraria from Kowa Company, Ltd. The other authors have no competing interests.

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**Authors’ contributions**

KN conceived the study and participated in its design and coordination. MY and KN drafted the manuscript. MY, KN, TM, MY, MK, KA, TK, HO, YO, and DM performed the experiments. IH were supervisors. All authors read and approved the final manuscript.

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Tables

Table 1. Effects of pitavastatin, pemafibrate or combination of pitavastatin and pemafibrate on BP, HR and blood measurements

| Group                  | ND-vehicle | HD-vehicle | HD-pitavastatin |
|------------------------|------------|------------|-----------------|
| Food                   | ND         | HD         | HD              |
| Treatment              | Vehicle    | Vehicle    | Pitavastatin    |
| Number of rats         | 5          | 9          | 10              |
| SBP (mmHg)             | 143 ± 2    | 194 ± 7*   | 197 ± 8         |
| HR (beats/min)         | 376 ± 11   | 402 ± 37   | 383 ± 19        |
| Blood measurements     |            |            |                 |
| Total bilirubin (mg/dL)| 0.01 ± 0.01| 0.01 ± 0.01| 0.03 ± 0.03     |
| Total cholesterol (mg/dL)| 57 ± 4  | 114 ± 19*  | 95 ± 26         |
| Triglyceride (mg/dL)   | 164 ± 26   | 143 ± 43   | 141 ± 54        |
| Glucose (mg/dL)        | 241 ± 20   | 236 ± 30   | 232 ± 22        |

BP, blood pressure; ND, normal diet; HD, high-salt and high-fat diet; SBP, systolic blood pressure; HR, heart rate. Values are mean ± SD. *P < 0.001 vs. ND-vehicle group. #P < 0.005 vs. HD-vehicle. §P < 0.001 vs. HD-pitavastatin group. †P < 0.01 vs. HD-pemafibrate group. ‡P < 0.005 vs. HD-vehicle. **P
< 0.001 vs. HD-vehicle. ## P < 0.05 vs. HD-vehicle. §§P < 0.01 vs. HD-vehicle.

Figures

Figure 1

Scheme of experimental protocol. We used 7-week-old male Dahl salt-sensitive rats. Rats were fed a normal diet (ND) (0.3% NaCl and 4.5% fat) or a high-salt and high-fat diet (HD) (8% NaCl and 29.4% fat). The rats were divided into five different groups and treated with vehicle, pitavastatin (0.3 mg/kg), pemafibrate (K-877) (0.5 mg/kg), or a combination of pitavastatin (0.3 mg/kg) and pemafibrate (K-877) (0.5 mg/kg) for 12 weeks. At 19 weeks, blood collection and evaluation of endothelium-dependent relaxations of thoracic aorta were performed.
Effects of pitavastatin, pemafibrate, or a combination of pitavastatin and pemafibrate on plasma cholesterol profile. Rats were fed a normal diet (ND) or a high-salt and high-fat diet (HD). The rats were divided into five groups and treated with vehicle, pitavastatin, pemafibrate (K-877), or a combination of pitavastatin and pemafibrate. A. Total cholesterol, B. chylomicron (CM)-cholesterol, C. very low-density lipoprotein (VLDL)-cholesterol, D. low-density lipoprotein (LDL)-cholesterol, E. high-density lipoprotein (HDL)-cholesterol. Data are expressed as the mean ± SD.
Effects of pitavastatin, pemafibrate, or combination of pitavastatin and pemafibrate on plasma triglyceride profile. Rats were fed a normal diet (ND) or a high-salt and high-fat diet (HD). The rats were divided into five groups and treated with vehicle, pitavastatin, pemafibrate (K-877), or combination of pitavastatin and pemafibrate. A. Total triglyceride (TG), B. chylomicron (CM)-TG, C. very low-density lipoprotein (VLDL)-TG, D. low-density lipoprotein (LDL)-TG, E. high-density lipoprotein (HDL)-TG. Data are expressed as the mean ± SD.
Effects of pitavastatin, pemafibrate or combination of pitavastatin and pemafibrate on endothelium-dependent vascular relaxations in response to acetylcholine. Rats were fed a normal diet (ND) or a high-salt and high-fat diet (HD). The rats were divided into five groups and treated with a vehicle, pitavastatin, pemafibrate (K-877), or combination of pitavastatin and pemafibrate. Data are expressed as the mean ± SD. *P < 0.001, HD-vehicle group versus ND-vehicle group. # P < 0.05, HD-combination group versus HD-vehicle group.
Figure 5

Effects of pitavastatin, pemafibrate, or combination of pitavastatin and pemafibrate on total and phospho-eNOS expression in the rat aorta. A. Images of Western blot analysis of total and phospho-endothelial nitric oxide synthase (eNOS) in aorta from rats fed a high-salt and high-fat diet (HD). B. Western blot analysis of total eNOS. C. Western blot analysis of phospho-eNOS. Data are expressed as the mean ± SD.