

Fenofibrate Modulates HO-1 and Ameliorates Endothelial Expression of Cell Adhesion Molecules in Systolic Heart Failure

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Background: Endothelial activation and dysfunction have been implicated in the pathogenesis and progression of heart failure (HF). In the present study, we investigated if endothelial expression of cell adhesion molecules (CAMs) is inhibited by fenofibrate, a peroxisome proliferator-activated receptor α (PPAR α) agonist with anti-inflammatory and vascular protective effects, through the regulation of heme oxygenase-1 (HO-1).

Methods: We recruited a total of 20 patients with advanced systolic HF and 20 healthy volunteers who all provided blood samples. Cultured human pulmonary artery endothelial cells (HPAECs) were treated with 70% sera obtained from study individuals, with or without pretreatment with fenofibrate. The endothelial expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and HO-1 were analyzed by mRNA expression and Western blot.

Results: Stimulation of cultured HPAECs with serum from HF patients significantly activated nuclear factor- κ B (NF- κ B) and increased VCAM-1 and ICAM-1 expression but attenuated HO-1 expression. Immunohistochemistry study also confirmed that CAMs were up-regulated, whereas HO-1 was down-regulated in HF patients. HO-1 small interfering RNA significantly suppressed HO-1 expression and exaggerated the HF serum-induced CAM expression, whereas HO-1 inducer cobalt protoporphyrin IX simultaneously stimulated HO-1 expression and suppressed CAM expression. Pretreatment with fenofibrate prevented the decrease of HO-1 expression and the activation of NF- κ B as well as the increase of CAM expression that induced by HF patient serum.

Conclusions: Our study demonstrated that fenofibrate may exert beneficial effects in patients with systolic HF through regulation of HO-1 expression and amelioration of endothelial activation.

Key Words: Cell adhesion molecules • Endothelial cells • Heart failure • Heme oxygenase-1 • Peroxisome proliferator-activated receptor- α

INTRODUCTION

Endothelial activation and dysfunction have been implicated in the pathogenesis and progression of heart failure (HF).¹⁻⁵ Markers of endothelial activation (selectins and cell adhesion molecules) and endothelial leukocyte adhesiveness were significantly increased in patients with advanced HF and those who develop pulmonary edema.⁶⁻¹¹ Significantly higher cell adhesion molecule (CAM) levels and endothelial leukocyte adhesiveness were associated with adverse clinical outcomes in HF patients.^{6-8,10,11}

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Expression of heme oxygenase-1 (HO-1) in the endothelium plays a cardioprotective role in HF.¹²⁻¹⁶ Heart failure may cause increased protein expression and enzymatic activity of HO-1 in the lung.¹⁷ Furthermore, HO-1 may inhibit the expression of CAMs associated with endothelial activation and suppress endothelial cell apoptosis.^{18,19}

Peroxisome proliferator-activated receptor α (PPAR α) deficiency may lead to impaired functional capacity of the heart.²⁰⁻²² Recent studies demonstrated numerous pleiotropic effects of fenofibrate, a PPAR α activator, on the heart that afford direct myocardial protection besides its lipid lowering effects.²³⁻³⁰ Our previous study also demonstrated that fenofibrate can inhibit endothelial monocyte adhesion in HF through inhibition of cytokine-induced CAM expression,³¹ suggesting that fenofibrate may ameliorate vascular inflammation and endothelial dysfunction and exert beneficial effects in HF patients.

It is well-known that many CAMs possess the regulatory sequences for the binding of nuclear factor-kappa B (NF- κ B), activator protein-1 (AP-1), and/or AP-2 in their promoter regions, just as HO-1³² and HO-1 can inhibit the expression of pro-inflammatory genes associated with endothelial activation.¹⁸ Furthermore, HO-1 expression is transcriptionally up-regulated by PPARs.³³ Therefore, in the present study, we sought to determine the effect of fenofibrate on the possible regulatory role of endothelial HO-1 in mediating the expression of CAMs and the functional importance of this process in patients, which have not previously been well studied.

METHODS

Study population

A total of 20 consecutive outpatients with stable advanced chronic HF were recruited from Cheng-Hsin General Hospital. Patients were included if they had New York Heart Association functional class II or III symptoms of HF, and a left ventricular ejection fraction (LVEF) of < 35% by echocardiography. The etiology of HF was determined as ischemic when coronary angiography revealed > 70% luminal diameter narrowing in at least 2 major epicardial coronary arteries. In those patients with HF without coronary artery disease for whom the endomyocardial biopsy revealed findings compatible

with dilated cardiomyopathy, the cause of HF was determined to be dilated cardiomyopathy.

Patients were excluded if they had hemodynamically significant obstructive valvular heart disease, cor pulmonale, restrictive or hypertrophic cardiomyopathy, myocarditis, constrictive pericarditis or congenital heart disease. Patients were excluded if there was severe comorbidity, or if there was evidence of systemic infection or an inflammatory illness. Those patients taking non-steroid anti-inflammatory drugs, antioxidants, L-arginine, or lipid-lowering agents such as statins and fibrates were also excluded.

Twenty age-matched healthy subjects provided blood samples for use as normal controls. No subject in the control group had any clinical signs or symptoms of HF and their LVEFs were all > 50% by echocardiography. Written informed consent was obtained from all participants, and the study protocol was approved by the local ethics committee.

Blood sampling and isolation of human serum

Blood samples were obtained from the forearm using standard vein puncture. All samples were placed immediately on ice, centrifuged at 4 °C within 2 hours, and then frozen to -20 °C and stored at that temperature until use. The time intervals between blood sampling and LVEF studies were all within one week.

Measurement of circulating levels of CAMs

Assays for circulating vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were done concurrently to minimize any effects of repeated freeze-thaw cycles. The levels of VCAM-1 and ICAM-1 were measured by means of enzyme-linked immunosorbent assay by commercial kits (R&D Systems, Inc., Minneapolis, MN, USA for ICAM-1 and Biosource International, Camarillo, CA, USA for VCAM-1). The intra-assay and inter-assay coefficients for VCAM-1 and ICAM-1 in our laboratory were approximately 5% and 10%, respectively.

Culture of HPAECs

Human pulmonary artery endothelial cells (HPAECs, Cascade Biologics, Portland, OR, USA) were grown in endothelial cell growth medium (medium 200, Cascade Biologics) in a poly-L-lysine-coated culture dish in a hu-

modified incubator with 5% CO₂ at 37 °C. The culture medium was renewed every 3 to 4 days. In all experiments, the cell passage number was between 3 and 6.

Isolation of total RNA and real-time PCR

Total RNA was isolated using a RNeasy Mini Kit and a RNase-free DNase set (Qiagen, Valencia, CA, USA). RNA (2 µg) was reverse-transcribed using the SuperScript™ First-Strand Synthesis System for reverse transcription polymerase chain reaction kit (Invitrogen, Carlsbad, CA, USA). The primers for real-time PCR were designed using Primer Express software (Real Quant, Roche) based on published sequences. The following primers were used in the present study: human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) – 5'-AGC CAC ATC GCT CAG ACA-3' (sense) and 5'-GCC CAA TAC GAC CAA ATC C-3' (antisense); human VCAM-1 – 5'-AGG GGA CCA CAT CTA CGC T-3' (sense) and 5'-ACA GAG CTC CCA TTC ACG A-3' (antisense); and human ICAM-1 – 5'-GGC AAG AAC CTT ACC CTA CG-3' and 5'-GAG ACC TCT GGC TTC GTC AG-3' (antisense). Procedures included an initial denaturation at 94 °C for 180 s, followed by 40 cycles at 95 °C for 30 s, 60 °C for 25 s, 72 °C for 30 s, and 1 cycle at 72 °C for 7 min. Fluorescence data were acquired at the end of amplification. A melt analysis was run for all products to determine the specificity of the amplification using the Real Quant software (Roche). All values were normalized to the constitutive expression of the housekeeping gene GAPDH.

Western blot analysis

The cell lysate was prepared using a cell lysis buffer (Cell Signaling Technology, Beverly, MA, USA) and Western blot analyses were performed. Briefly, the cell lysate (25 to 40 µg) was subjected to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes, which was then blotted. After being blocked with 5% skim milk in Tween-20/phosphate-buffered saline (PBS), blots were incubated with various primary antibodies, including anti-human VCAM-1, ICAM-1, HO-1, and α-tubulin (Chemicon, Temecula, CA, USA). Blots were then incubated with the horseradish peroxidase-conjugated secondary antibodies. The signal was detected using Chemiluminescence Reagent Plus (NEN, Boston, MA, USA). The intensity of each band was

scanned and quantified using a densitometer linked to computer software (ImageQuant; Amersham, Amersham, UK).

Nuclear extracts preparation and electrophoretic mobility shift assay (EMSA)

The following are protocols for nuclear protein extracts preparation. Briefly, after washing with PBS, the cells were scraped off the plates in 0.6 ml of ice-cold buffer A (10 mM N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) (HEPES), pH 7.9, 10 mM KCl, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 1.5 mM MgCl₂, and 2 µg/ml each of aprotinin, pepstatin, and leupeptin). After centrifugation at 300 g for 10 min at 4 °C, the cells were resuspended in buffer B (80 µl of 0.1% Triton X-100 in buffer A), left on ice for 10 min, then centrifuged at 12,000 g for 10 min at 4 °C. The nuclear pellets were resuspended in 70 µl of ice-cold buffer C (20 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 0.42 M NaCl, 1 mM DTT, 0.2 mM EDTA, 1 mM PMSF, 25% glycerol, and 2 µg/ml each of aprotinin, pepstatin, and leupeptin), then incubated for 30 min at 4 °C, followed by centrifugation at 15,000 g for 30 min at 4 °C. The resulting supernatant was stored at -70 °C as the nuclear extract. Protein concentrations were determined by the Bio-Rad method.

EMSA was performed according to DIG gel shift kit instruction manual (Roche, USA). Gel shift oligonucleotides were labeled with terminal transferase and DIG-11-dUTP (Roche, USA).

Immunohistochemical staining

Human coronary arteries of 3 recipient hearts obtained from 3 dilated cardiomyopathy patients underwent heart transplantation (2 males and one female, aged 25, 48 and 52 years, respectively) and one carotid artery from one healthy male donor (aged 18 years) were used in immunohistochemical study.

The vessels were rinsed with ice-cold PBS, immersion-fixed in 4% buffered paraformaldehyde, paraffin-embedded, and then cross-sectioned for further study. The arterial sections were deparaffinized, rehydrated, and washed with PBS, then non-specific binding was blocked by pre-incubation for 1 h at room temperature with PBS containing 5 mg/ml of bovine serum albumin. Sequential serial sections were incubated with goat

anti-human von Willebrand factor (vWF, a marker of endothelial integrity), HO-1, VCAM-1, and ICAM-1 primary antibody (R&D systems, U.S.A.). The sections were then incubated with biotinylated conjugated horse anti-goat IgG for 1 h at room temperature and antigen-antibody complexes detected by incubation with avidin-biotin-horseradish peroxidase complex for 1.5 h at room temperature, followed by 0.5 mg/ml of 3,3'-diaminobenzidine/0.01% hydrogen peroxide in 0.1 M Tris-HCl buffer, pH 7.2, as chromogen (Vector Lab, USA). Negative controls were performed by non-specific primary antibody IgG.

The effects of small interfering RNA (siRNA) for HO-1 and HO-1 inducer cobalt protoporphyrin-IX (COPPIX) on endothelial activation

The siRNA nucleotide sequences for human HO-1 and control GAPDH were designed and synthesized using the computer software and Silencer™ siRNA construction kit from Ambion (Austin, TX, USA) according to the manufacturer's instructions. HPAECs grown in 100-mm dishes were transfected with HO-1 or GAPDH siRNA at selected concentrations with the use of Oligofectamine reagent (Invitrogen) in a total transfection volume of 6 ml medium. After incubation at CO₂ at 37 °C, 5% CO₂ for 5 hours, 3 ml of normal growth medium was added and incubated with HPAECs for 48 hours.

Exposure of HPAECs to COPPIX, a synthetic protoporphyrin that induces the expression of HO-1 (10 μM; Sigma, Saint Louis, MO, USA) for 12 h was used to increase the expression of HO-1 to measure the inhibitory effect of HO-1 on endothelial activation.

Pharmacological treatments with fenofibrate

Fenofibrate was obtained from Laboratories Fournier S.A. (Fontaine Les Dijon, France) and was dissolved in dimethyl sulfoxide (DMSO) as a stock solution. The confluent HPAECs were pretreated with the growth medium supplemented with 50 μM fenofibrate for 18 hours, followed by 70% sera obtained from patients with HF or normal individual for 12 hours at 37 °C. The endothelial expression of ICAM-1, VCAM-1, HO-1, and transcriptional activation of NF-κB was then confirmed by mRNA expression and Western blot, and EMSA, respectively.

Statistics

All values were expressed as mean ± standard error

of mean. Comparisons of clinical and biochemical characteristics between two groups were made with the Wilcoxon rank-sum test for quantitative data and with Fisher's exact test for qualitative data. Comparisons between multiple groups were determined by means of a one-way analysis of variance (ANOVA) followed by Dunnett's test. A p value of less than 0.05 was considered statistically significant.

RESULTS

Baseline clinical characteristics and the circulating levels of CAMs of the study patients

The baseline characteristics of the 20 patients with advanced HF and the 20 healthy controls are shown in Table 1.

Significant differences in LVEF, systolic blood pressure, and heart rates were detected between the patients with HF and normal controls. There were more men than women in this sample. Patients were included with both ischemic as well as non-ischemic etiology in the HF group.

Induction of CAMs on HPAECs by serum obtained from HF patients

Incubation for 12 h of HPAECs with serum from HF patients (HFS) markedly increased the protein and mRNA expression of VCAM-1 and ICAM-1 as compared with those incubated with serum from normal subjects (NRS) and control medium (Figure 1A and B). Although the protein and mRNA expression of VCAM-1 was also elevated to some extent by serum from normal subjects, the ICAM-1 was not significantly elevated. Moreover, the induction of both VCAM-1 and ICAM-1 by serum from HF patients was evident as early as 3 h, and the augmentation lasted for at least 24 h, whereas serum from normal subjects only time-dependently induced VCAM-1 expression to a lesser extent (Figure 1C).

Down-regulation of HO-1 expression by serum obtained from HF patients

Incubation for 12 h of HPAECs with 70% serum from HF patients significantly decreased the protein and mRNA expression of HO-1 as compared with those incu-

Table 1. Baseline clinical characteristics of the study population

	Normal control (n = 20)	Heart failure (n = 20)	p value
Age (yrs)	63 ± 3	66 ± 5	NS
Male, n (%)	16 (80%)	15 (75%)	NS
LVEF (%)	63 ± 5	28 ± 3	< 0.001
Ischemic heart disease (%)	-	5 (25)	-
Hypertension (%)	-	12 (60)	-
Diabetes mellitus (%)	-	8 (40)	-
Smoking (%)	-	6 (30)	-
Alcohol use (%)	-	2 (10)	-
Systolic blood pressure (mmHg)	126 ± 4	112 ± 5	< 0.01
Heart rates (bpm)	69 ± 3	95 ± 6	< 0.01
Medications			
Diuretic	-	18 (90%)	-
Digitalis	-	12 (60%)	-
ACEI/ARB therapy	-	19 (95%)	-
Vasodilator therapy	-	14 (70%)	-
Beta-blockers	-	13 (65%)	-
Aspirin	-	6 (30%)	-
VCAM-1 (ng/ml)	441.9 ± 32.8	590.7 ± 70.1	< 0.05
ICAM-1 (ng/ml)	180.2 ± 25.2	250.5 ± 35.2	< 0.05

ACEI/ARB, angiotensin converting enzyme inhibitors or angiotensin II receptor blockers; ICAM-1, intercellular adhesion molecule-1; LVEF, left ventricular ejection fraction; NS, non-significant; VCAM-1, vascular cellular adhesion molecule-1.

bated with serum from normal subjects and control medium (Figure 2A). The down-regulation of HO-1 expression by serum from HF patients was evident at 12 h, and the HO-1 expression decreased further at 24 h, whereas serum from normal subjects did not significantly affect HO-1 expression (Figure 2B).

Immunohistochemical staining for HO-1 and CAMs (Figure 2C)

In vessels of normal subjects, HO-1 protein was detected predominantly in the endothelial vWF-positive cells, relative to the thin layer of endothelial cells. In vessels of patients with HF, HO-1 protein expression was much attenuated in the vWF-positive cells in the endothelium. By contrast, increased expression of VCAM-1 and ICAM-1 was presented mainly in both endothelium and tunica media of vessels from HF patients.

Modulation of HF serum-induced CAM expression via HO-1 regulation

As shown in Figure 3A, HO-1 siRNA dose-dependently attenuated HO-1 expression. Attenuation of HO-1 by HO-1 siRNA potentiated the induction of VCAM-1 and ICAM-1 expression by serum from HF patients and normal subjects (Figure 3B).

HPAECs were exposed to CoPPiX dose (2.5-20 μ M)-

and time (3-24 h)-dependently induced high levels of HO-1 expression (Figure 3C). The increased endothelial expression of VCAM-1 by serum from HF patients and normal subjects and the expression of ICAM-1 by serum from HF patients were significantly inhibited by CoPPiX (Figure 3D).

Fenofibrate inhibits HF serum-induced CAM expression and HO-1 down-regulation and attenuates serum-induced activation of NF- κ B

HPAECs were pretreated for 18 h with fenofibrate before the addition of serum from HF patients and normal subjects. Fenofibrate caused a dose-dependent decrease in VCAM-1 expression induced by serum from HF patients and normal subjects, and dose-dependently decreased HF serum-induced ICAM-1 expression (Figure 4A). By contrast, fenofibrate treatment at 25 and 50 μ M significantly inhibited HF serum-induced HO-1 down-regulation (Figure 4B).

Gel shift assays showed that HF serum treatment resulted in strong activation of NF- κ B, whereas serum from normal subjects only slightly increased the shifted band, compared to control medium. Pretreatment with fenofibrate reduced the density of the NF- κ B shifted bands induced by serum both from HF patients and normal subjects (Figure 4C).

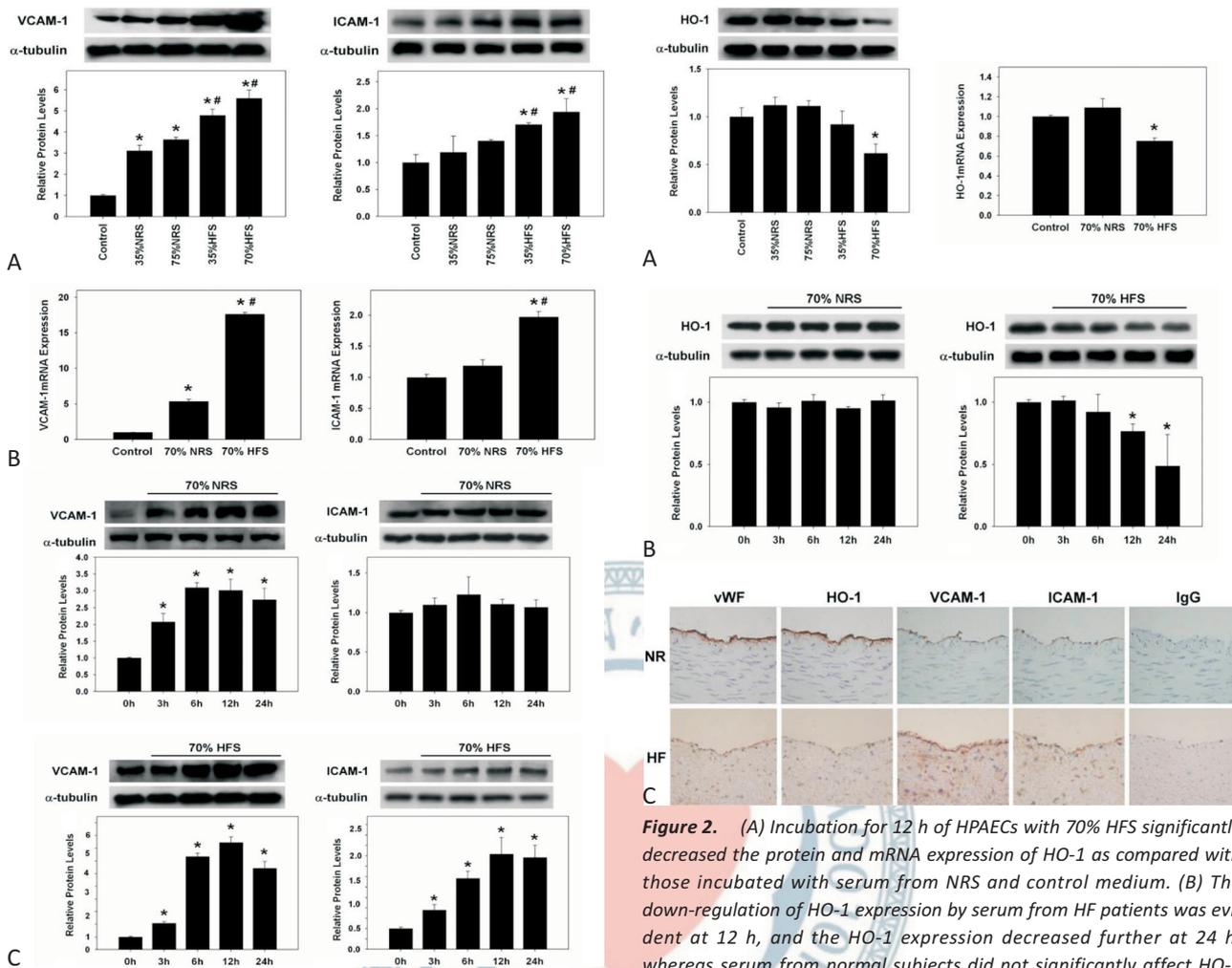


Figure 1. Induction of adhesion molecules and down-regulation of heme-oxygenase I (HO-1) expression on human pulmonary artery endothelial cells (HPAECs) by serum obtained from HF patients. (A and B) Incubation for 12 h of HPAECs with serum from HF patients (HFS) markedly increased the protein and mRNA expression of VCAM-1 and ICAM-1 as compared with those incubated with serum from normal subjects (NRS) and control medium (Control). (C) The induction of both VCAM-1 and ICAM-1 by serum from HF patients was evident as early as 3 h, and the augmentation lasted for at least 24 h, whereas serum from normal subjects only time-dependently induced VCAM-1 expression to a lesser extent. Densitometric analysis was conducted with software to semiquantify Western blot data. Three independent experiments gave similar results. * $p < 0.05$, compared to control group; # $p < 0.05$, compared to NRS group.

DISCUSSION

In the present study, we showed that stimulation of cultured HPAECs with HF patient serum significantly down-regulated HO-1 expression, activated redox-sensitive transcription factor NF- κ B, and increased VCAM-1

Figure 2. (A) Incubation for 12 h of HPAECs with 70% HFS significantly decreased the protein and mRNA expression of HO-1 as compared with those incubated with serum from NRS and control medium. (B) The down-regulation of HO-1 expression by serum from HF patients was evident at 12 h, and the HO-1 expression decreased further at 24 h, whereas serum from normal subjects did not significantly affect HO-1 expression. Three independent experiments gave similar results. The summarized data (mean \pm standard error of mean) from 3 separate experiments is shown in the bar graph. * $p < 0.05$, compared to control group. (C) Immunohistochemical analysis revealed the distribution of vWF, HO-1, VCAM-1, and ICAM-1 in the arteries from the normal subject and HF patients. In vessel of the normal subject (NR), HO-1 protein was detected predominantly in the endothelial vWF-positive cells, relative to the thin layer of endothelial cells. In vessels of patients with heart failure (HF), HO-1 protein expression was much attenuated in the vWF-positive cells in the endothelium. By contrast, increased expression of VCAM-1 and ICAM-1 was presented mainly in both endothelium and tunica media of vessels from HF patients.

and ICAM-1 expression. Immunohistochemistry study of coronary and carotid arteries confirmed that VCAM-1 and ICAM-1 were up-regulated, whereas HO-1 was down-regulated in HF patients. HO-1 inducer CoPPiX simultaneously stimulated HO-1 expression and suppressed VCAM-1 and ICAM-1 expression, whereas HO-1 siRNA significantly exaggerated HF patient serum-induced

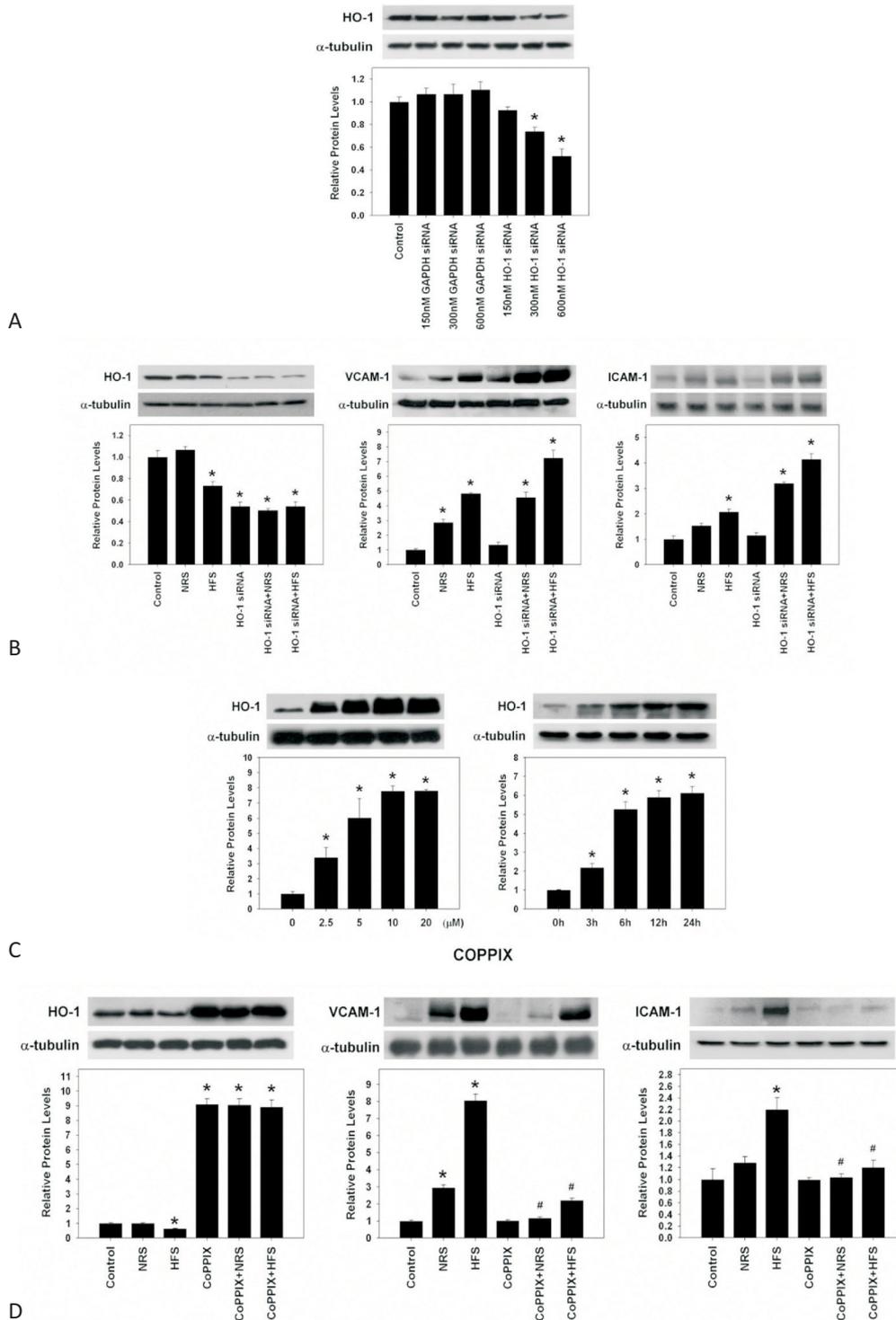


Figure 3. Modulation of HF serum-induced adhesion molecules expression via HO-1 regulation. (A) HO-1 small interfering RNA (siRNA) dose-dependently attenuated HO-1 expression. (B) Attenuation of HO-1 by HO-1 siRNA potentiated the induction of VCAM-1 and ICAM-1 expression by HFS and NRS. (C) Exposure of HPAECs to CoPPiX dose (2.5-20 μ M)- and time (3-24 h)-dependently induced high levels of HO-1 expression. (D) The increased endothelial expression of VCAM-1 by serum from HF patients and normal subjects and the expression of ICAM-1 by serum from HF patients were significantly inhibited by CoPPiX. Three independent experiments gave similar results. The summarized data (mean \pm SEM) from 3 separate experiments is shown in the bar graph. * $p < 0.05$, compared to control group; # $p < 0.05$, compared to NRS/HFS group.

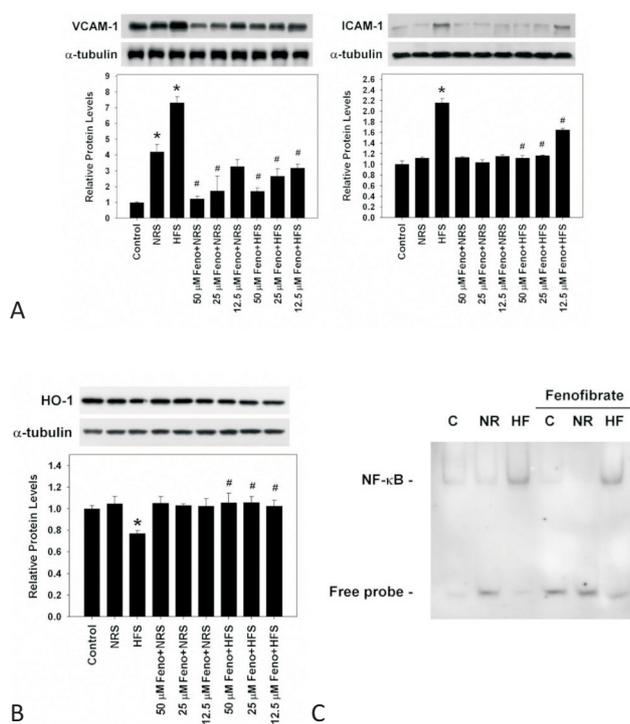


Figure 4. Fenofibrate prevents HF serum-induced HO-1 down-regulation and inhibits adhesion molecule expression. HPAECs were pretreated for 18 h with PPAR- α agonist fenofibrate (Feno) before addition of serum from HF patients (HFS) and normal subjects (NRS). (A) Fenofibrate (12.5, 25, and 50 μ M) caused a dose-dependent decrease in VCAM-1 expression induced by serum from HF patients and normal subjects, and dose-dependently decreased HF serum-induced ICAM-1 expression. (B) Fenofibrate treatment at 25 and 50 μ M significantly inhibited HF serum-induced HO-1 down-regulation. Three independent experiments gave similar results. (C) Gel shift assays showed that HFS treatment resulted in the strongly activation of NF- κ B, whereas NRS only slightly increase the shifted band, compared to control medium. Pretreatment with fenofibrate reduced the density of the NF- κ B shifted bands induced by serum both from HF patients and normal subjects. The summarized data (mean \pm SEM) from 3 separate experiments is shown in the bar graph. * p < 0.05, compared to control group; # p < 0.05, compared to NRS/HFS group.

VCAM-1 and ICAM-1 expression on HPAECs. Furthermore, pretreatment with fenofibrate may prevent the decrease of HO-1, the activation of NF- κ B, as well as the increase of VCAM-1 and ICAM-1 induced by HF serum.

Endothelial activation and dysfunction may contribute to exercise intolerance, impaired myocardial perfusion, left ventricular remodeling, cardiogenic shock, and pulmonary edema.^{1-5,34,35} CAMs induced by inflammatory cytokines may play a direct and potentially critical role in the pathophysiology of HF.^{6-11,34,35} We and others have reported that patients with advanced HF

have elevated circulating levels of CAMs, irrespective of the cause of HF.⁶⁻⁸ Significantly higher circulating CAM levels and endothelial leukocyte adhesiveness were predictors of adverse clinical outcomes in HF patients.^{6-8,10,11}

HO-1 exhibits anti-inflammatory property to provide endothelial protection during atherogenesis, restenosis, and other inflammatory cardiovascular disorders.^{12-16,36,37} Moreover, HO-1 may inhibit the expression of CAMs associated with endothelial activation via a mechanism that is associated with the inhibition of NF- κ B activation¹⁸ and suppress endothelial cell apoptosis via the activation of p38 MAPK.¹⁹ Recently, it has been demonstrated that HO-1 expression is transcriptionally regulated in human endothelial cells and vascular smooth muscle cells by PPAR α , indicating a mechanism of anti-inflammatory action of PPAR ligands via up-regulation of HO-1.³³ In this study, our results further confirmed that the endothelial activation and vascular inflammatory processes are systemic and can be modified through regulation of HO-1 in HF.

Beyond its regulatory effects on cardiac energy and control of myocardial lipid metabolism,^{20,24} PPAR α also exert numerous effects by interaction with different transcription factors to repress pro-inflammatory genes.^{24,25,29} The activators of PPAR α have been demonstrated to exert cardiovascular antioxidant and anti-inflammatory effects by interfering negatively with transcription factor pathways such as NF- κ B, signal transducers and activators of transcription (STAT), and AP-1.^{24,25,29} Among these, the transcription factor NF- κ B is critical for the induction of the VCAM-1 and ICAM-1 examined in this study. Furthermore, fenofibrate has an additional potential to prevent the induction and progression of hypertensive heart damage, cardiac hypertrophy, heart failure, myocarditis, lipotoxic cardiomyopathy and vascular endothelial dysfunction-associated cardiovascular abnormalities.²⁶⁻³⁰ Our results support this hypothesis because fenofibrate counteracted HO-1 down-regulation and suppressed endothelial CAM expression that were induced by pro-inflammatory stimuli of HF serum via inhibition of NF- κ B signaling pathway. Since a daily dosage of fenofibrate 200 mg produced plasma concentrations within the range of 5-35 mg/l (14-100 μ M) in 12 dyslipidaemic patients receiving the drug over a 3-month period,³⁸ the doses we studied in this experiment are clinically relevant.

CONCLUSION

In conclusion, although our study does not exclude a role for the effects of PPAR α activators on the expression of other factors involved in endothelial activation and leukocyte-endothelial interaction, nor does it rule out the possibility that the molecules tested may act through unrelated mechanisms in addition to PPAR α activation, this work does identify HO-1 as a target gene for PPAR α and provides a basis for further investigation of HO-1 modulation by fenofibrate as a therapeutic strategy for endothelial activation and dysfunction in HF as well.

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DISCLOSURES

None.

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