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Biochemical Effects of Some Peroxisome Proliferator Activated Receptor Ligands in Hyperhomocysteinemic-rats

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ABSTRACT

Hyperhomocysteinemia (HHcy) is implicated in peripheral vascular, cerebrovascular and coronary heart disease. This study was conducted to investigate the effects of pioglitazone (PPAR-γ agonist) and gemfibrozil (PPAR-α agonist), either alone or in combination, in HHcy-rats. HHcy was induced by keeping rats on high methionine diet (8% w/w) for 6 weeks. Rats were divided into two major groups; normal fed rats and methionine overload rats. Each group was further subdivided into four subgroups: a) control group, b) pioglitazone (10 mg/kg), c) gemfibrozil (100 mg/kg), and d) combination of the chosen PPAR ligands. The drugs were given orally daily for consecutive six weeks. The results showed that the great reduction of HHcy was obtained by the drug combination. Induction of HHcy in rats resulted in elevation of LDL-cholesterol and suppression of HDL-cholesterol. Hepatic paraoxonase-1 activity was inhibited in all HHcy-rat groups. RT-PCR for liver cyclooxygenase-2 (COX-2) showed marked expression in HHcy-rats, which was attenuated by treatment with PPAR agonists. RT-PCR for cystathionine β-synthase (CBS) showed positive expression in all HHcy-rat groups except that treated with combination of PPAR ligands. These results suggest that pioglitazone-gemfibrozil combination showed ameliorative effect on hyperhomocysteinemia and its consequences in rats.

INTRODUCTION

Peroxisome proliferator activated receptors (PPARs) are nuclear lipid-activated transcription factors that regulate the expression of genes involved in the control of lipid and lipoprotein metabolism, glucose homeostasis and inflammatory processes. Their wide range of potential therapeutic actions makes them attractive for the development of oral agents targeting risk factors associated with the metabolic syndrome, type 2 diabetes and cardiovascular diseases (Michalik *et al*., 2006). The PPAR subfamily of nuclear receptors includes three isotypes, namely PPAR-α, PPAR-γ and PPAR-β/δ, which are encoded by three distinct genes. Each isotype displays distinct patterns of tissue distribution and has specific pharmacological activators (Trifilieff *et al*., 2003). All marketed PPAR-α agonists belong to the fibrate class. They are widely prescribed as hypolipidemic agents to reduce triglycerides while increasing plasma HDL-cholesterol. Moreover, they reduce vascular inflammation and thrombogenicity (Barbier *et al*., 2002). The thiazolidinediones (TZDs) are PPAR-γ agonists, which are used as antidiabetic and insulin sensitizers improving insulin mediated glucose uptake (Gastaldelli *et al*., 2006). Although the metabolic effects of the PPAR ligands have been well studied, there is a lack of data on their effects on tHcy. Hyperhomocysteinemia is considered an independent risk factor for vascular disorders resulting from atherosclerosis (Ueland & Hustad, 2008). Hyperhomocysteinemia is associated with aging, cystathionine beta synthase deficiency, methionine synthase deficiency, 5,10-methylenetetrahydrofolate reductase deficiency and decreased vitamin cofactors like folate, vitamin B_{12}, and B_6 (Hayden & Tyagi, 2004). Several potential mechanisms for Hcy-induced atherosclerosis have been proposed. These include decreased bioavailability and synthesis of endothelium-derived nitric oxide, the vascular production of superoxide, increased proliferation of smooth muscle cells, enhanced coagulability, and protein modification. Additionally, it has been suggested that Hcy may contribute to atherosclerosis by lipid dysregulation (Stangl *et al*., 2007). Oxidative stress has been implicated in HHcy. Homocysteine promotes oxidant injury to vascular cells, particularly the endothelium and the eNOS enzyme reaction, through the autoxidation of Hcy, formation of Hcy-mixed disulfides, interaction of Hcy thiolactones, and protein homocysteinylation (Jakubowski *et al*., 2000).

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Because some PPAR agonists were demonstrated to have beneficial effects in some inflammatory as well as diabetic complications and cardiovascular disorders, the biochemical effects and the exact mechanisms of PPAR ligands in rats with HHcy need to be clarified.

This study was conducted to investigate the effects of pioglitazone (PPAR-γ agonist) and gemfibrozil (PPAR-α agonist) either alone or in combination on tHcy, inflammation, lipid profile and oxidative stress in HHcy-rats. Also, RT-PCR of CBS and COX-2 in livers had been studied.

MATERIALS AND METHODS

Materials

Carrageenan, gemfibrozil, paraoxon-ethyl, sulfanilamide, sodium nitrite and vanadium chloride were purchased from Sigma-Aldrich Chemical Company, USA. N-(1-naphthyl)ethylenediamine was obtained from Aldrich Chemical Co. Ltd, England. Pioglitazone was a gift from Medical Union Pharmaceuticals Abu-Sultan, Ismailia, Egypt. Other chemicals and reagents were all of high analytical grade.

Animals

This study included about 130 male albino rats, 80-100 g each. Rats were purchased from the animal house of Geiza Institute of Ophthalmology, Cairo, Egypt. Rats were weighed and housed in wire cages for two weeks under identical environmental conditions for acclimatization. The animals were allowed free access to balanced laboratory diet and tap water *ad libitum*.

Experimental Design

Rats were weighed and randomly divided into three major groups; *Group A* (Control groups; n=40), *Group B* (Hyperhomocysteinemic groups; n=40) and *Group C* (Carrageenan groups; n=50). *Group A* and *C* were maintained on normal balanced diet for six weeks. Hyperhomocysteinemia was induced in *Group B* by keeping rats on methionine-rich diet (8% methionine) for six weeks. Each group was further divided into ***four*** subgroups, representing vehicle control, pioglitazone (10 mg/kg), gemfibrozil group (100 mg/kg), and pioglitazone (10 mg/kg) + gemfibrozil (30 mg/kg) group. In *Group A* and *B*, the drugs were given orally daily to rats for consecutive six weeks at the start of the experiment. *Group C* was subjected to carrageenan injection (0.1 mL of 1.5% solution) in the right plantar surface of the hind paw. The drugs were given at the specified doses once orally to rats 1 hour prior to carrageenan injection. The feet of rats were cut off 4 hours after carrageenan injection and weighed.

At the end of the six weeks, rats of *Groups A* and *B* were anaesthetized by ether and blood was withdrawn by cardiac puncture into tubes containing $EDTANa_2$. Blood samples were centrifuged, plasma was divided into 3 aliquots, and stored at -20 ^oC. Rats were sacrificed and the livers were excised, washed twice with ice-cold saline, divided into two portions and kept frozen at - 80 ^oC.

Methods

Plasma glucose (Trinder, 1969), triglycerides (Fossati & Prencipe, 1982), total-cholesterol (Allain *et al*., 1974), and HDL-C (Allain *et al*., 1974). LDL-C was calculated using the formula of Friedewald *et al.* (1972). Plasma nitric oxide was determined according to the method of Miranda *et al.* (2001).

Plasma total homocysteine

The first step in determining Hcy includes reduction and derivatisation. During the reduction, Hcy is cleaved from albumin and homocystine is reduced into two homocysteines. The derivatisation reagent transforms Hcy into a fluorescent product, which is then measured by HPLC (Waters, USA) with scanning fluorescence detector (Waters 474, USA) using kits obtained from Immundiagnostik Co. Ltd., Germany.

Hepatic paraoxonase-1 activity

Liver samples (200 mg) were homogenized in sucrose solution (1:4), centrifuged at 12.500 rpm for 10 min, and the supernatant was obtained. Paraoxonase-1 activity was determined by measuring the initial rate of hydrolysis of paraoxon (o,o-diethyl-o-p-nitrophenyl phosphate) to p-nitrophenol, which absorbance was monitored at 405 nm (Beltowski *et al.*, 2002).

RT-PCR analysis of CBS and COX-2

Total RNA was extracted from rat liver tissue by TRIzol® reagent obtained from Invitrogen Co., according to Chomczynski & Sacchi (1987). Total RNA (1 μg) was reverse-transcribed and amplified on thermal cycler (Bio-RAD, USA) and the synthesized cDNA was used directly for amplification by PCR. Specific primers for CBS (Li *et al.*, 2006) and COX-2 (Jones *et al.*, 1999) were used to generate 93 bp and 253 bp, respectively. The primers and amplification conditions were listed in Table (1). PCR products were detected by agarose (1.5%) gel electrophoresis and visualized by ethidium bromide staining.

Table. 1: Primers and amplification conditions for RT-PCR analysis.

Primer (base pair)	Sequence	Amplification Conditions
CBS (93 bp) Sense: Antisense:	5'-ATTCCCCACATTACCAC-3' 5'-TTGATTCTGACCATAGG-3'	**1 cycle:** 42 ^oC, 60 min, **1 cycle:** 95 ^oC, 5 min, **35 cycles:** 95 ^oC, 30 sec-55 ^oC, 1.5 min-72 ^oC, 1.5 min-72 ^oC, 10 min
COX-2 (253 bp) Sense: Antisense:	5'-TGGTGCCGGGTCTGATGATG-3' 5'-GCAATGCGGTTCTGATACTG-3'	**1 cycle:** 42 ^oC, 60 min, **1 cycle:** 95 ^oC, 5 min, **35 cycles:** 95 ^oC, 30 sec-60 ^oC, 1.5 min-72 ^oC, 1.5 min-72 ^oC, 10 min

Statistical Analysis

The obtained data were analyzed using Minitab computer software (version 13). The results were expressed as the mean ± SEM. Comparison between different groups was carried out by one way analysis of variance (ANOVA) using Tukey-Kramer test. The correlations between parameters within groups were done using Microsoft Excel 2003 computer software. The level of significance was set at $P < 0.05$.

Table. 2: Effect of pioglitazone and/or methionine overload on body weight difference, plasma tHcy, plasma glucose, lipid profile, hepatic PON1 activity and plasma NO:

Parameter	*Normal Control*	*Met*	*Pio*	*Pio+Met*
Weight Difference (g)	79.80± 5.60	44.10 ^{a**}± 4.40	118.90 ^{a**}± 7.60	86.40 ^{b**c**}± 4.50
Plasma Homocysteine (µM)	9.10± 1.40	132.20 ^{a**}± 7.50	9.35± 0.99	142.70 ^{a**c**}± 5.10
Plasma Glucose (mg/dL)	108.2± 3.3	111.1± 3.5	99.9± 4.5	116.7± 3.6
Plasma Cholesterol (mg/dL)	57.02± 2.60	63.01± 3.50	61.80± 3.90	54.03± 3.2
Plasma Triglycerides (mg/dL)	70.62± 3.40	64.60± 4.10	75.70± 4.90	65.02± 2.40
Plasma HDL-C (mg/dL)	14.07± 1.20	10.50 ^{a*}± 0.83	36.98 ^{a**b**}± 1.70	30.64 ^{a**b**}± 3.30
Plasma LDL-C (mg/dL)	28.85± 2.10	39.58 ^{a*}± 3.00	9.67 ^{a**b**}± 2.30	8.52 $^{a** b**}$± 2.40
Liver PON1 Activity (U/g)	864 ± 34	526 ^{a**} ± 34	1064 ^{a**} ± 27	502 ^{a**c**} ± 26
Plasma NO (µM)	11.39± 0.73	18.98 ^{a*}± 1.50	7.72 ^{a*}± 1.20	11.13 ^{b*} ±1.60

Data are presented as mean ± SEM; n=8 for each group; Met: Methionine (8% in diet), Pio: Pioglitazone (10 mg/kg, orally). a: Significant *versus* normal control group, b: Significant *versus* Met group, c: Significant *versus* Pio group, *: P<0.05, **: P<0.01.

Table. 3: Effect of gemfibrozil and/or methionine overload on body weight difference, plasma tHcy, plasma glucose, lipid profile, hepatic PON1 activity and plasma NO:

Parameter	*Normal Control*	*Met*	*Gem*	*Gem +Met*
Weight Difference (g)	79.80± 5.60	44.10 ^{a**}± 4.40	86.60± 6.20	55.30 ^{a*c**}± 6.10
Plasma Homocysteine (µM)	9.10± 1.40	132.20 ^{a**}± 7.50	10.85± 1.00	128.30 ^{a**c**}± 4.30
Plasma Glucose (mg/dL)	108.2± 3.3	111.1± 3.5	107.8± 4.0	116.1± 3.8
Plasma Cholesterol (mg/dL)	57.02± 2.60	63.01± 3.50	74.87 ^{a**}± 3.20	69.20 ^{a*}± 4.00
Plasma Triglycerides (mg/dL)	70.62± 3.40	64.60± 4.10	46.60 ^{a**}± 5.20	77.62 ^{b*c**}± 2.80
Plasma HDL-C (mg/dL)	14.07± 1.20	10.50 ^{a*}± 0.83	31.80 ^{a**b**}± 4.00	39.90 ^{a**b**}± 2.40
Plasma LDL-C (mg/dL)	28.85± 2.10	39.58 ^{a*}± 3.00	33.8± 4.60	13.80 ^{a*b**c**}± 4.50
Liver PON1 Activity (U/g)	864 ± 34	526 ^{a**} ± 34	706 ^{a**} ± 23	265 ^{a**b**c**}± 16
Plasma NO (µM)	11.39± 0.73	18.98 ^{a*}±1.50	14.02± 1.40	14.59 ^{a*b*}± 1.10

Data are presented as mean ± SEM; n=8 for each group; Met: Methionine (8% in diet), Gem: Gemfibrozil (100 mg/kg, orally). a: Significant *versus* normal control group, b: Significant *versus* Met group, c: Significant *versus* Gem group, *: P<0.05, **: P<0.01.

Table. 4: Effect of pioglitazone/gemfibrozil combination and/or methionine overload on body weight difference, plasma tHcy, plasma glucose, lipid profile, hepatic PON1 activity and plasma NO:

Parameter	*Normal Control*	*Met*	*Pio+Gem*	*Pio+Gem+Met*
Weight Difference (g)	79.80± 5.60	44.10 ^{a**}± 4.40	96.63 ^{a*}± 3.20	91.00 ^{b**}± 6.30
Plasma Homocysteine (µM)	9.10± 1.40	132.20 ^{a**}± 7.50	13.04± 1.03	89.70 ^{a**b**c**}± 4.90
Plasma Glucose (mg/dL)	108.2± 3.3	111.1± 3.5	99.1± 5.0	98.8± 5.1
Plasma Cholesterol (mg/dL)	57.02± 2.60	63.01± 3.50	61.20± 3.80	63.00± 3.90
Plasma Triglycerides (mg/dL)	70.62± 3.40	64.60± 4.10	56.70 ^{a*}± 3.90	58.30 ^{a*}± 4.40
Plasma HDL-C (mg/dL)	14.07± 1.20	10.50 ^{a*}± 0.83	46.10 ^{a**b**}± 3.20	46.81 ^{a**b**}± 2.40
Plasma LDL-C (mg/dL)	28.85± 2.10	39.58 ^{a*}± 3.00	3.74 ^{a**b**}± 1.20	4.57 ^{a**b**}± 1.40
Liver PON1 Activity (U/g)	864 ± 34	526 ^{a**} ± 34	953 ± 22	268 ^{a**b**c**} ± 19
Plasma NO (µM)	11.39± 0.73	18.98 ^{a*}± 1.50	8.28 ^{a*}±1.00	7.31 ^{a*b*}± 1.50

Data are presented as mean ± SEM; n=8 for each group; Met: Methionine (8% in diet), Pio: Pioglitazone (10 mg/kg), Gem: Gemfibrozil (30 mg/kg, orally). a: Significant *versus* normal control group, b: Significant *versus* Met group, c: Significant *versus* Pio+Gem group, *: P<0.05, **: P<0.01.

Table. 5: Correlation study between plasma total homocysteine (tHcy) and different parameters in control groups and HHcy-rats treated with pioglitazone and gemfibrozil:

Parameter	Plasma tHcy (µM) in control groups	Plasma tHcy (µM) in HHcy-rats treated with Pio/Gem
Plasma HDL-C (mg/dL)	r = -0.607, P<0.02	r = -0.745, P<0.01
Plasma LDL-C (mg/dL)	r = 0.735, P<0.01	r = 0.772, P<0.01
Liver PON1 Activity (U/g)	r = -0.889, P<0.01	r = 0.729, P<0.01
Plasma NO (µM)	r = 0.672, P<0.01	r = 0.511, P<0.05

RESULTS

Table (2) shows the effect of pioglitazone on normal and HHcy-rat groups. Compared to normal control group, Met overload significantly decreased body weight (P<0.01), HDL-C (P<0.05) and liver PON1 activity (P<0.01), but significantly increased plasma tHcy (P<0.01), LDL-C (P<0.05) and plasma NO (P<0.05). On the other hand, pioglitazone treatment to normal rats significantly increased body weight (P<0.01), HDL-C (P<0.01) and hepatic PON1 activity (P<0.01) in comparing with normal controls. Pioglitazone treatment to normal rats also decreased LDL-C (P<0.01) and plasma NO (P<0.05). In HHcy-rats, pioglitazone treatment increased body weight (P<0.01) and HDL-C (P<0.01), while decreased LDL-C (P<0.01) and plasma NO (P<0.05), compared to methionine overload group. Pioglitazone treatment to HHcy-rats did not significantly alter plasma tHcy and hepatic PON1 in comparing with methionine fed group.

Table (3) shows the effect of gemfibrozil on normal and HHcy-rat groups. Compared to normal control group, gemfibrozil treatment significantly increased plasma total cholesterol (P<0.01) and HDL-C (P<0.01), while significantly decreased plasma TGs (P<0.01) and LDL-C (P<0.01). On the other hand, gemfibrozil

administered to HHcy-rats increased plasma TGs (P<0.05) and HDL-C (P<0.01), but decreased LDL-C (P<0.01), hepatic PON1 activity (P<0.01) and plasma NO (P<0.05) in comparing with methionine overload rat group.

Table (4) shows the effect of pioglitazone/gemfibrozil combination on normal and HHcy-rat groups. Compared to normal control group, co-treatment with pioglitazone and gemfibrozil significantly increased body weight (P<0.05) and HDL-C (P<0.01), while significantly decreased plasma TGs (P<0.05), LDL-C (P<0.01) and plasma NO (P<0.05). Compared to methionine overload rat group, pioglitazone/gemfibrozil combined treatment significantly increased body weight (P<0.01) and HDL-C (P<0.01), but significantly decreased plasma tHcy (P<0.01), LDL-C (P<0.01), hepatic PON1 activity (P<0.01) and plasma NO (P<0.05).

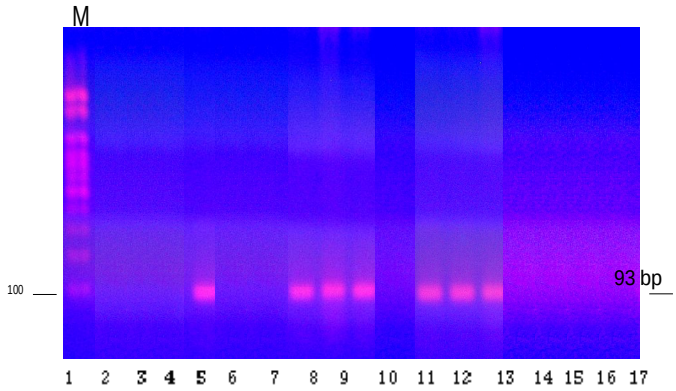

Fig. 1: Gel electrophoresis of RT-PCR for hepatic CBS in all rat groups. Each lane represents pooled sample of 3-5 rats liver; n=8-9 rats for each group. Total RNA was extracted from rat liver samples, reverse transcribed, and amplified using specific rat CBS primers to yield 93 bp. M: 100 bp ladder DNA marker. Lane 1,2,3: Control Normal, Lane 4: Met, Lane 5: Pio, Lane 6: Gem, Lane 7,8,9: Pio+Met, Lane 10: Gem+Pio, Lane 11,12,13: Gem+Met, Lane 14,15,16,17: Pio+Gem+Met.

Figure (1) shows gel electrophoresis of RT-PCR for hepatic CBS. The results show that CBS gene was expressed in liver of HHcy rat group (Lane 4), and HHcy rats treated with either pioglitazone (Lane 7,8,9) or with gemfibrozil (Lane 11,12,13).

On the other hand, HHcy rats treated with drug combination did not show expression of CBS gene (Lane 14,15,16,17). No apparent expression of CBS gene was observed in liver of normal control rats (Lane 1,2,3) or normal rats treated with pioglitazone (Lane 5), gemfibrozil (Lane 6) or both drugs (Lane 10).

Figure (2) shows gel electrophoresis of RT-PCR for hepatic COX-2. Positive COX-2 gene expression was observed in 75% of liver samples of methionine overload control rats (Lane 4,5,6), in 33% of pioglitazone treated methionine fed rats (Lane 10), in 33% of gemfibrozil treated normal fed rats (Lane 9), and 50% of combined treatment methionine fed rats (Lane 14). In control and other rat groups, COX-2 gene expression was negative.

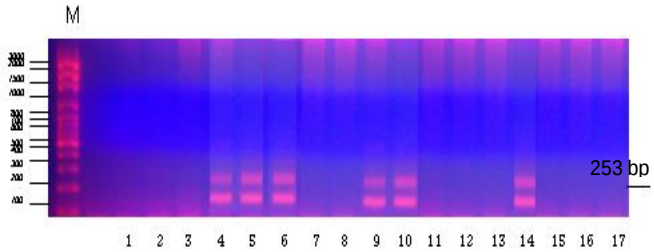

Fig. 2: Gel electrophoresis of RT-PCR for hepatic COX-2 in all rat groups. Each lane represents pooled sample of 3-5 rats liver; n=8-9 rats for each group. Total RNA was extracted from rat liver samples, reverse transcribed, and amplified using specific rat COX-2 primers to yield 253 bp. M; 100 bp ladder DNA marker. Lane 1,2: Control Normal, Lane: 3,4,5,6: Met, Lane 7,8,9: Gem+Met, Lane 10,11,12: Pio+Met, Lane 13: Pio+Gem, Lane 14,15: Pio+Gem+Met, Lane 16: Pio, Lane 17: Gem.

DISCUSSION

This study performed to clarify the effects of pioglitazone (PPAR-γ agonist) and/or gemfibrozil (PPAR-α agonist) on plasma tHcy, gene expression of hepatic CBS and cyclooxygenase-2 (COX-2), lipid profile, hepatic paraoxonase-1 (PON1), and plasma nitric oxide (NO) in rats with induced HHcy. The obtained results revealed that HHcy was induced in rats kept on methionine-rich diet (8%) for six weeks. Homocysteine level in methionine overload rats was about 14.5 times its value in normal control rat group.

It was observed that induction of HHcy in rats by methionine overload resulted in significant decrease in body weight by about 45% compared to control normal rats. This loss in the body weight was in agreement with the study of Velez-Carrasco and coworkers (2008), which revealed that there was a marked reduction in the body weight of mice kept on 20% methionine for eight weeks.

The loss of the body weight of HHcy-rats was ameliorated with pioglitazone treatment. The weight gain with pioglitazone treatment may be ascribed to thiazolidinediones-induced fluid retention (Miyazaki *et al.*, 2002). Treatment of diabetic patients with thiazolidinediones (TZDs) is found to be associated with increased adipogenesis and fat redistribution, i.e., an increase in subcutaneous adipose tissue and a concomitant decrease in visceral fat (Akazawa *et al.*, 2000).

Furthermore, HHcy-rats received both pioglitazone and gemfibrozil did not exhibit weight loss, which may be attributed to pioglitazone rather than to gemfibrozil, because gemfibrozil monotherapy to HHcy rats did not prevent weight loss.

Treatment of carrageenan-inflamed rats with pioglitazone and/or gemfibrozil produced marked anti-inflammatory effect. The % inhibition of rat-paw edema was ≈ 33% by pioglitazone, ≈ 19% by gemfibrozil, and ≈ 30% by pioglitazone/gemfibrozil combination. A mechanism associated with the anti-inflammatory and anti-edematogenic activities of PPAR-α and PPAR-γ agonists might be the inhibition of the synthesis or the release of some inflammatory mediators.

These results were consistent with that obtained by RT-PCR of COX-2, which revealed that pioglitazone and/or gemfibrozil administered to HHcy-rats ameliorated methionine-

induced expression of COX-2 in rat liver. The present work showed that HHcy resulted in expression of COX-2 in 75% of methionine overload rats. Each of pioglitazone and gemfibrozil exhibited expression of COX-2 in only one third of animals, whereas pioglitazone/gemfibrozil combination produced gene expression of COX-2 in about half of the rat livers.

In the present study, the induced HHcy was alleviated in rat group co-treated with pioglitazone and gemfibrozil; where plasma tHcy level was about 68% of its value in methionine overload rats. On the other hand, either drug alone did not significantly affect plasma tHcy level.

Gemfibrozil given to rats in the present study did not affect tHcy level compared to methionine or normal control group. Gemfibrozil is the only fibrate that does not induce HHcy (Westphal *et al.*, 2001).

The results of this study revealed that PCR of the gene expression of CBS in the liver of the experimental rat groups showed a positive marked expression of CBS in all rat groups kept on methionine overload diet for induction of HHcy. This induced expression of CBS is considered as normal accommodation of the methionine overload and HHcy. Thus, HHcy could induce the expression of CBS to counteract the deleterious effect of the elevated tHcy levels by transsulfuration in all rat groups fed on high methionine diet either treated with PPARs agonist or not.

The observed marked expression of CBS gene in rat liver was abolished by co-treatment with pioglitazone and gemfibrozil, which was accompanied by reduced level of plasma tHcy. Thus, it can be suggested that the catabolism of homocysteine is enhanced by transcriptional regulation of hepatic cystathionine β-synthase when HHcy is developed and these changes are prevented by correction of plasma tHcy.

The present results indicated that HHcy was associated with decreased activity of paraoxonase-1 (PON1) in rat livers. PON1 activity in methionine overload rats was about 61% its value in normal controls. These results were in agreement with previous reports indicating that PON1 activity was decreased with a plasma tHcy level greater than 15 µM in mice (Hamelet *et al.*, 2007).

Moreover, gemfibrozil administered to normal rats produced a decrease in liver PON1 activity and its value become about 82% of its value in normal controls. The present data indicated a decrease in liver PON1 activity in all rat groups treated with gemfibrozil.

This effect of gemfibrozil on PON1 activity may be explained by gemfibrozil-induced increase in plasma HDL-C concentration, which may saturate HDL pool. Gemfibrozil could be also metabolized by the liver to give a potent antioxidant metabolite; 5-(4-hydroxy-2,5-dimethyl-phenoxy)-2,2-dimethyl pentanoic acid, which was found to protect against lipoprotein oxidation (Kawamura *et al.*, 2000).

On the contrary, pioglitazone given to normal rats for 6 weeks produced a significant increase in liver PON1 activity compared to normal controls. This may be attributed to the pioglitazone-induced increase in plasma HDL-C as shown in the present work. Pioglitazone increased the level of plasma HDL-C to about 2.6 fold and liver PON1 activity to about 1.2 fold of their corresponding values in normal control.

Although pioglitazone monotherapy given to normal rats showed an increase in the liver PON1 activity, the co-treatment with pioglitazone and gemfibrozil to normal rats resulted in insignificant change in the activity of liver PON1. This could be explained by the effect of pioglitazone, which markedly enhance and increase PON1 activity that counteracts the lowering effect of gemfibrozil.

The deleterious effect of elevated plasma tHcy on HDL-C could be explained by the finding that Hcy reduces the levels of apo A-I protein and inhibits the synthesis of Apo A-I in rats' hepatocytes (Liao *et al.*, 2006).

The increased effect of pioglitazone on HDL could be attributed to stimulation of *de novo* hepatic synthesis of apo A-I without affecting the uptake of HDL-C. It was suggested that pioglitazone mediated hepatic activation of PPAR-α may be one of the mechanisms of action of pioglitazone to elevate HDL-C (Qin *et al.*, 2007).

Gemfibrozil is also effective in increasing HDL-C levels *via* a PPAR-α dependent mechanism. The increase in plasma HDL-C depends partly on an overexpression of apo A-I and apo A-II (Fruchart & Duriez, 2006).

Hyperhomocysteinemia does not influence expression of genes involved in cholesterol homeostasis; the relative mRNA concentrations of sterol regulatory element-binding protein 2 (SREBP-2), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and LDL-receptor. Thus, HHcy does not alter the concentration of total cholesterol in plasma, circulating lipoproteins, liver, and intestine (Stangl *et al.*, 2007).

The increase of LDL-C in HHcy-rats has been previously reported (Veerkamp *et al.*, 2003). Homocysteine promotes free radical oxidation of LDL particles and also promotes LDL modification by formation of LDL-C-Hcy-thiolactone aggregates, increasing the risk of CVD (Earnest *et al.*, 2002).

In the present study, pioglitazone treatment to normal rats or HHcy-rats produced a significant reduction of plasma LDL-C, compared to respective control groups. It was found by Leonhardt *et al.* (2008) that pioglitazone improves insulin sensitivity and glycemic control and appear to lower the concentration of atherogenic small dense LDL-C particles. The plasma cholesterol and triglyceride concentrations remained unchanged.

On the other hand, gemfibrozil given to normal rats increased the level of plasma total cholesterol and LDL-C compared to normal control group. These results may be interpreted by the finding that gemfibrozil decreased bile acid synthesis and increased biliary cholesterol secretion which were associated with reduced cholesterol absorption efficiency and increased synthesis of cholesterol and saturation of bile. Thus, risk of gallstone formation could increase (Vanhanen & Miettinen, 1995). The increased plasma LDL-C in gemfibrozil treated group may be related to the increased level of plasma total cholesterol.

When gemfibrozil was given to HHcy rats, it showed insignificant change in plasma total cholesterol, compared to HHcy control group. Elevation of HDL-C without change of plasma total cholesterol in HHcy rats treated with gemfibrozil may account for the decreased level of plasma LDL-C observed in the present study. Similar results were obtained in normal and HHcy rat groups co-treated with pioglitazone and gemfibrozil, where total plasma cholesterol was increased and it associated with an increase of HDL and a decrease of LDL.

Measurement of plasma triglycerides in the present work revealed that induction of HHcy in rats did not significantly affect plasma TGs. The hypolipidemic effect of gemfibrozil was evident; gemfibrozil lowered plasma TGs in normal rats treated with gemfibrozil alone or pioglitazone/gemfibrozil combination.

This hypotriglyceridemic effect of gemfibrozil is thought to be due to a PPAR-α dependent stimulation of lipoprotein lipase and of apolipoprotein (apo) A-V and to an inhibition of apo C-III expression, thereby increasing lipoprotein triglyceride lipolysis (Fruchart & Duriez, 2006).

Unfortunately, gemfibrozil administered to HHcy rats produced a significant increase in plasma TGs compared to HHcy control group. These results could be attributed to the elevated levels of both Hcy and cholesterol in Gem/Met group. Zulli and his coworkers (1998) reported that both the elevated levels of Hcy and cholesterol resulting in further elevation of plasma TGs levels, which further enhances the risk of coronary artery disease. Thus, HHcy and hypercholesterolemia counteract the hypolipidemic effect of gemfibrozil.

Accordingly, treatment of HHcy-rats with pioglitazone and gemfibrozil attenuate the synergistic effect of both HHcy and hypercholesterolemia on the TGs levels of this group.

On the other hand, pioglitazone given to normal rats or HHcy-rats did not alter plasma TGs level. These results could be supported by the foundation of Shimono *et al.* (2001), who suggested that pioglitazone reported no change in postprandial TGs in type 2 diabetic patients. The results of the present data showed that treatment of normal rats with pioglitazone or with pioglitazone plus gemfibrozil resulted in a significant reduction in plasma NO level compared to normal controls.Some investigators reported that PPAR-γ agonists can form a heterdimer with retinoid-X receptor. The activation of PPAR-γ:RXR acts to suppress the promoter activity of the NF-κB and activator protein-1 transcriptional activity, with the consequent suppression of iNOS and TNF-α production (Uchimura *et al.*, 2001).

In conclusion, the present study revealed that HHcy represents a risk factor for cardiovascular disease; it induced an increase in plasma LDL-C, a decrease in plasma HDL-C and an increase in plasma NO. Moreover, liver PON1 activity was greatly suppressed. The combination of both PPAR-γ (pioglitazone) and PPAR-α (gemfibrozil) agonists ameliorated HHcy and was effective in lowering plasma tHcy in methionine overload rats. This drug combination also produced a favorable elevation of HDL-C, reduction of LDL-C and reduction of NO in plasma. Besides their use as insulin sensitizer (pioglitazone) and antihyperlipidemic (gemfibrozil) drugs, these PPAR agonists may have an additional benefit in reducing the risk of cardiovascular disease in type 2 diabetes mellitus and other circulatory disorders. The effects of PPAR agonists on HHcy and risk factors of cardiovascular disease deserve further investigations.

REFERENCES

Akazawa S, Sun F, Ito M, Kawasaki E and Eguchi K. Efficacy of troglitazone on body fat distribution in type 2 diabetes. Diabetes Care, 2000; 23: 1067-1071.

Allain CC, Poon LS, Chan CSG, Richmond W and FU PC. Enzymatic determination of total serum cholesterol. Clin Chem, 1974; 20(4): 470-475.

Barbier O, Torra IP, Duguay Y, Blanquart C, Fruchart JC, Glineur C and Staels B. Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. Arterioscler Thromb Vasc Biol, 2002; 22: 717-726.

Beltowski J, Wojcicka G and Marciniak A. Species and substrate specific stimulation of human plasma paraoxonase 1 (PON1) activity by high chloride concentration. Acta Bioch Polon, 2002; 49(4): 927-936.

Chomczynski P and Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem, 1987; 162(1): 156-159.

Earnest C, Cooper KH, Marks A and Mitchell TL. Efficacy of a complex multivitamin supplement. Nutrition, 2002; 18(9): 738-742.

Fossati P and Principe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem, 1982; 28: 2077-2080.

Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem, 1972; 18(6): 499-502.

Fruchart JC and Duriez P. Mode of action of fibrates in the regulation of triglyceride and HDL-cholesterol metabolism. Drugs Today, 2006; 42(1): 39-64.

Fruchart JC and Duriez P. Mode of action of fibrates in the regulation of triglyceride and HDL-cholesterol metabolism. Drugs Today, 2006; 42(1): 39-64.

Gastaldelli A, Ferrannini E, Miyazaki Y, Matsuda M, Mari A and Defronzo RA. Thiazolidinediones improve beta-cell function in type 2 diabetic patients. Am J Physiol Endocrinol Metab, 2006; 292: E871-E883.

Hamelet J, Ait-Yahya-Graison E, Matulewicz E, Noll C, Badel-Chagnon A, Camproux AC, Demuth K, Paul JL, Delabar JM and Janel N. Homocysteine threshold value based on cystathionine beta synthase and paraoxonase 1 activities in mice. Eur J Clin Invest, 2007; 37(12): 933-938.

Hayden MR and Tyagi SC. Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis: The pleiotropic effects of folate supplementation. Nutr J, 2004; 3(1): 4.

Jakubowski H, Zhang L, Bardeguez A and Aviv A. Homocysteine thiolactone and protein homocysteinylation in human endothelial cells: implications for atherosclerosis. Circ Res, 2000; 87(1): 45-51.

Jones MK, Sasaki E, Halter F, Pai R, Nakamura T, Arakawa T, Kuroki T and Tarnawski AS. HGF triggers activation of the COX-2 gene in rat gastric epithelial cells: action mediated through the ERK2 signaling pathway. FASEB J, 1999; 13: 2186-2194.

Kawamura M, Miyazaki S, Teramoto T, Ashidate K, Thoda H, Ando N and Kaneko K. Gemfibrozil metabolite inhibits *in vitro* low-density lipoprotein (LDL) oxidation and diminishes cytotoxicity induced by oxidized LDL. Metabolism, 2000; 49(4): 479-485.

Leonhardt W, Pfutzner A, Muller J, Pietzsch J, Forst T, Karagiannis E, Lubben G and Hanefeld M. Effects of pioglitazone and/or simvastatin on low density lipoprotein subfractions in non-diabetic patients with high cardiovascular risk: A sub-analysis from the PIOSTAT Study. Atherosclerosis, 2008; 201(1): 155-162.

Li N, Chen L, Muh RW and Li PL. Hyperhomocysteinemia associated with decreased renal transsulfuration activity in dahl S rats. Hypertension, 2006; 47: 1094-1100.

Liao D, Tan H, Hui R, Li Z, Jiang X, Gaubatz J, Yang F, Durante W, Chan L, Schafer AI, Pownall HJ, Yang X and Wang H. Hyperhomocysteinemia decreases circulating high-density lipoprotein by inhibiting apolipoprotein A-I protein synthesis and enhancing HDL cholesterol clearance. Circ Res, 2006; 99: 598-606.

Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, Grimaldi PA, Kadowaki T, Lazar MA, O'Rahilly S, Palmer CN, Plutzky J, Reddy JK, Spiegelman BM, Staels B and Wahli W. International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. Pharmacol Rev, 2006; 58(4): 726-741.

Miranda KM, Espey MG and Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 2001; 5(1): 62-71.

Miyazaki Y, Mahankali A, Matsuda M, Mahankali S, Hardies J, Cusi K, Mandarino LJ and DeFronzo RA. Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab, 2002; 87: 2784-2791.

Qin S, Liu T, Kamanna VS and Kashyap ML. Pioglitazone stimulates apolipoprotein A-I production without affecting HDL removal in HepG2 cells: involvement of PPAR-alpha. Arterioscler Thromb Vasc Biol, 2007; 27(11): 2428-2434.

Shimono D, Kuwamura N, Nakamura Y and Koshiyama H. Lack of effect of pioglitazone on postprandial triglyceride levels in type 2 diabetes. Diabetes Care, 2001; 24(5): 971.

Stangl GI, Weisse K, Dinger C, Hirche F, Brandsch C and Eder K. Homocysteine thiolactone-induced hyperhomocysteinemia does not alter concentrations of cholesterol and SREBP-2 target gene mRNAs in rats. Exp Biol Med, 2007; 232: 81-87.

Stangl GI, Weisse K, Dinger C, Hirche F, Brandsch C and Eder K. Homocysteine thiolactone-induced hyperhomocysteinemia does not alter concentrations of cholesterol and SREBP-2 target gene mRNAs in rats. Exp Biol Med, 2007; 232: 81-87.

Trifilieff A, Bench A, Hanley M, Bayley D, Campbell E and Whittaker P. PPAR-α and -γ but not -δ agonists inhibit airway inflammation in a murine model of asthma: *in vitro* evidence for an NF-κB-independent effect. Br J Pharmacol, 2003; 139: 163-171.

Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem, 1969; 6: 24-28.

Uchimura K, Nakamuta M, Enjoji M, Irie T, Sugimoto R, Muta T, Iwamoto H and Nawata H. Activation of retinoic X receptor and peroxisome proliferator-activated receptor-γ inhibits nitric oxide and tumor necrosis factor-α production in rat Kupffer cells. Hepatology, 2001; 33(1): 91-99.

Ueland PM and Hustad S. Homocysteine and folate status in an era of folic acid fortification: balancing benefits, risks, and B-vitamins. Clin Chem, 2008; 54: 779-781.

Vanhanen HT and Miettinen TA. Cholesterol absorption and synthesis during pravastatin, gemfibrozil and their combination. Atherosclerosis, 1995; 115: 135-146.

Veerkamp MJ, de Graaf J, den Heijer M, Blom HJ and Stalenhoef AFH. Plasma homocysteine in subjects with familial combined hyperlipidemia. Atherosclerosis, 2003; 166(1): 111-117.

Velez-Carrasco W, Merkel M, Twiss C and Smith J. Dietary methionine effects on plasma homocysteine and HDL metabolism in mice. J Nutr Bio, 2008; 19(6): 362-370.

Westphal S, Dierkes J and Luley C. Effects of fenofibrate and gemfibrozil on plasma homocysteine. Lancet, 2001; 358: 39-40.

Zulli A, Buxton B, Doolan L and Liu JJ. Effect of homocysteine and cholesterol in raising plasma homocysteine, cholesterol and triglyceride levels. Life Sci, 1998; 62(24): 2191-2194.

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