ORIGINAL ARTICLES

Rosuvastatin and Metformin Decrease Inflammation and Oxidative Stress in Patients With Hypertension and Dyslipidemia

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Introduction and objectives. Both hypertension and dyslipidemia raise the risk of cardiovascular disease because they have proinflammatory effects and increase oxidative stress. The aim of this study was to evaluate the effects of rosuvastatin and metformin on inflammation and oxidative stress in patients with hypertension and dyslipidemia.

Methods. This open parallel-group clinical study involved 48 patients with hypertension and dyslipidemia. Of these, 16 were treated with rosuvastatin, 10 mg/day, while 16 received metformin, 1700 mg/day, and the 14 in the control group received starch placebo, 10 mg/day. The following variables were recorded during the study: age, weight, body mass index, blood pressure, glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNFα), glutathione reductase (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD).

Results. Administration of 10 mg/day of rosuvastatin decreased total cholesterol by 41.7%, LDL cholesterol by 63.0%, and triglycerides by 10.7%, and increased HDL cholesterol by 6.3%. Pharmacological treatment with either rosuvastatin or metformin lead to reductions in IL-6, TNFα, GSH and GPx levels and an increase in the SOD level, and there were significant interactions between the two treatment groups for these variables.

Conclusions. Rosuvastatin improved the lipid profile.

Moreover, both rosuvastatin and metformin reduced inflammation and oxidative stress. These results demonstrate the presence of an additional cardioprotective effect, which may result from a direct mechanism of action or be a pleiotropic effect. Further long-term studies are required to determine whether rosuvastatin or metformin can be used to decrease the cardiovascular risk resulting from oxidative stress and inflammation.

Key words: *Oxidative stress. Inflammation. Drugs. Pleiotropic effects. Cardiovascular risk.*

Rosuvastatina y metformina reducen la inflamación y el estrés oxidativo en pacientes con hipertensión y dislipemia

Introducción y objetivos. La hipertensión arterial (HTA) y la dislipemia incrementan el riesgo de enfermedad cardiovascular a través de los efectos proinflamatorios y el estrés oxidativo. Nuestro objetivo fue estimar el efecto de la rosuvastatina y la metformina en la inflamación y el estrés oxidativo en pacientes con HTA y dislipemia.

Métodos. En un ensayo clínico abierto paralelo, se estudió a 48 pacientes con HTA y dislipemia. Se trató a 16 pacientes con rosuvastatina 10 mg/día, 16 con metformina 1.700 mg/día y 16 con 10 mg de almidón como control. Las variables analizadas durante el estudio fueron edad, peso, índice de masa corporal (IMC), presión arterial, glucosa, colesterol total (CT), de las lipoproteínas de baja densidad (cLDL) y de las lipoproteínas de alta densidad (cHDL), triglicéridos (TG), interleucina 6 (IL-6), factor de necrosis tumoral alfa (TNFα), glutatión reductasa (GSH), glutatión peroxidasa (GPx) y superóxido dismutasa (SOD).

Resultados. Con 10 mg/día de rosuvastatina, disminuyeron el CT (41,7%), el cLDL (63%) y los TG (10,7%) y se incrementó el cHDL (6,3%). Después del tratamiento farmacológico con rosuvastatina o metformina, se encontró disminución e interacción entre grupos en la IL-6, el TNFα, la GSH y la GPx e incremento en la SOD.

Conclusiones. La rosuvastatina mejoró el perfil de lípi-

dos. Ambos fármacos reducen la inflamación y el estrés oxidativo. Estos resultados demuestran un efecto adicional cardioprotector, como un mecanismo de acción directo o a través de sus efectos pleiotrópicos. Son necesarios estudios adicionales a largo plazo para determinar si la rosuvastatina o la metformina serán fármacos útiles para disminuir el riesgo cardiovascular causado por el estrés oxidativo y la inflamación.

Palabras clave: *Estrés oxidativo. Inflamación. Fármacos. Efectos pleiotrópicos. Riesgo cardiovascular.*

ABBREVIATIONS

HBP: high blood pressure
HDL-C: high density lipoprotein cholesterol
IL-6: interleukin 6
LDL-C: low density lipoprotein cholesterol
SOD: superoxide dismutase
TNFα: tumor necrosis factor alpha

INTRODUCTION

In México, the prevalence of non-transmissible chronic disease, such as high blood pressure (HBP) and diabetes mellitus, has grown exponentially over the last 2 decades. Indeed, it is now more prevalent than transmissible disease. The prevalence of HBP has reached 30.1%[1] and is one of the main risk factors associated with cerebrovascular and coronary heart disease. It is thought that some 1.5% of all patients with HBP die each year for reasons directly associated with this problem.[1,2]

Some 36.5% of all Mexican patients with HBP also suffer dyslipidemia.[1] This complication increases the risk of cardiovascular disease. One of the possible mechanisms behind this lies in the proinflammatory effects of interleukin-6 (IL-6) and tumor necrosis factor (TNFα). A number of studies have shown that both cytokines are involved in the associated chronic vascular inflammatory reponse.[3-5] Inflammation is a source of oxidative stress, which is also involved in the development of atherosclerosis and HBP. Several studies indicate the importance of a change in the balance of oxidative and antioxidant enzymes in the progression of atherosclerosis, HBP, and diabetes mellitus type 2.[6-8]

The additional actions of drugs that reduce the serum concentration of lipids (statins)[9-11] and improve sensitivity to insulin (metformin)[12] are known as pleiotropic effects. These include (among others) the improvement of endothelial function (via an anti-inflammatory and antioxidant action), the stabilization of atherosclerotic plaques, and a reduction in the thrombogenic response.[13-15] This has allowed some of the mechanisms of oxidative stress regulation, in which oxidative and antioxidant enzymes take part, to be elucidated. However, a great deal of the information available is from laboratory experiments, and much more work is required to clarify the clinical significance of these drugs and their actions. The aim of this work was to determine the effects of rosuvastatin and metformin on oxidative stress in patients with HBP and dyslipidemia.

METHODS

This open, parallel group study was performed between July and September 2006. The initial study subjects were 510 patients with HBP and dyslipidemia selected from outpatients attending the Family Medicine Unit Nº. 80 of the Instituto Mexicano del Seguro Social (IMSS) in Morelia, Michoacán, México. Of these, 244 were excluded since they had concomitant diabetes mellitus 2, and a further 206 did not meet inclusion requirements since they were receiving pharmacological treatment for their dyslipidemia or had been prescribed an excluded anti-hypertension treatment. Twelve patients declined to participate.

The inclusion criteria were: *a)* to have HBP (≥130/85 mm Hg) and dyslipidemia (LDL-C ≥100 mg/dL, triglycerides ≥150 mg/dL, HDL-C <40 mg/dL in men, or <50 mg/dL in women)[16]; *b)* to be receiving no pharmacological treatment for dyslipidemia; *c)* to be receiving treatment for HBP with angiotensin converting enzyme inhibitors (ACEi); and *d)* to be ≥65 years o age.

Among the 48 patients who were finally included, none had modified their pharmacological treatment for HBP, their diet, nor their physical activity routine in the 3 months prior to inclusion. No changes were made during follow-up. The subjects were randomly assigned to 3 pharmacological intervention groups. Sixteen patients received 10 mg/day rosuvastatin orally with their evening meal (group GRos); 16 received metformin 1700 mg/day, administered as 2 tablets of 850 mg (in the first week 1 tablet/day was provided at breakfast and if tolerated this dose was increased to 1 tablet every 12 h) (group GMetf]); and 16 received a starch placebo 10 mg/day (control group [GC]). Treatment lasted 12 weeks (Figure 1). The minimum required sample size was estimated using the clinical trial equation[17]; the result was required to provide a confidence level of 95%, and an 80% power to detect a change in the serum IL-6 concentration of 0.6 pg/mL (standard deviation 0.5 pg/mL). The equation showed 13 patients per group were necessary. Sixteen were included in each to make up for any possible losses during follow-up.

The patient variables recorded at the time of inclusion were: age, body weight, height, body mass index (Quetelet index), number of years with HBP, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), LDL-C, HDL-C, triglycerides (TG), serum concentrations of the inflammation markers IL-6 and
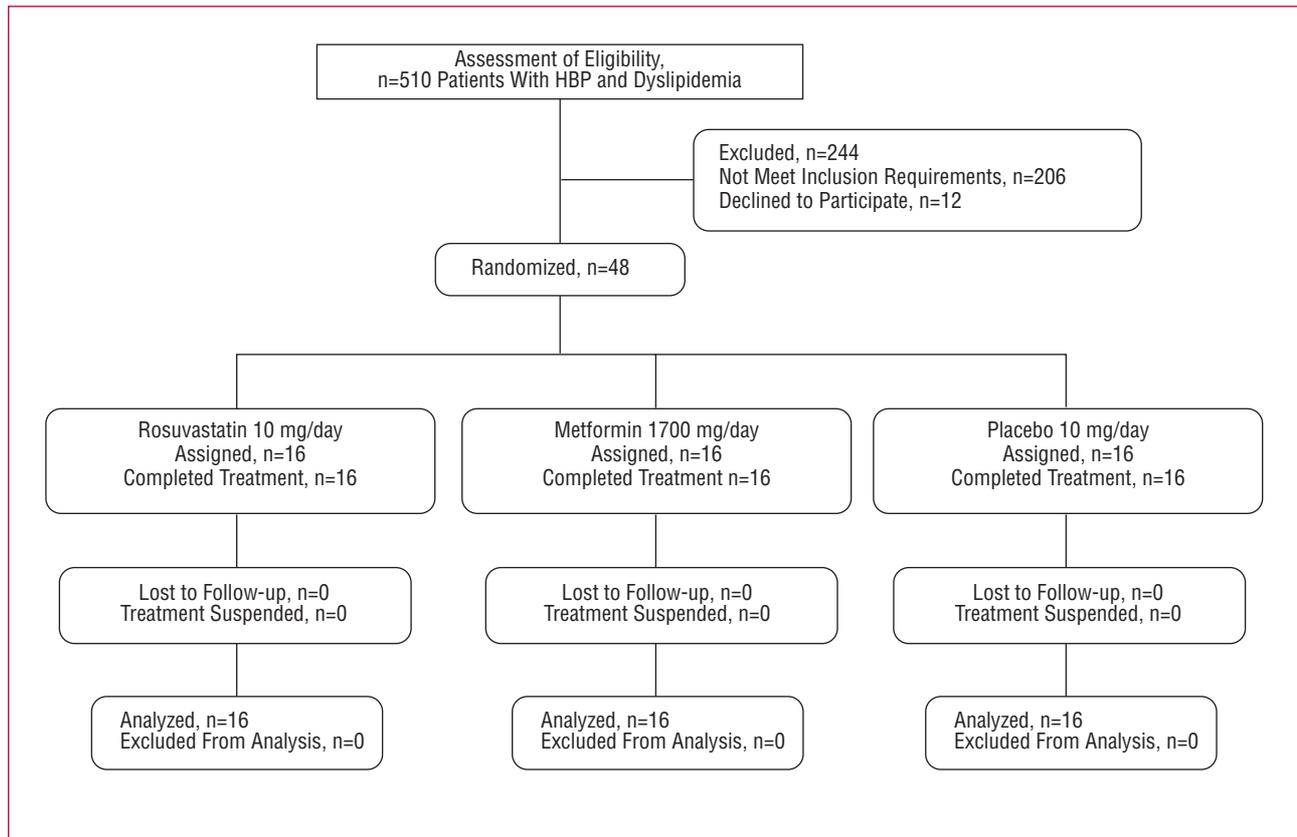
Figure 1. Progress of patients through the study process.

TNFα, and oxidative stress (activities of the enzymes glutathione reductase [GSH], glutathione peroxidase [GPx], and superoxide dismutase [SOD]). Patients attended a monthly appointment at the Epidemiological Research Unit, Clínica del Hospital General Regional Nº. 1 IMSS to check for any signs of adverse effects of treatment, to check adherence to treatment (via counting of the pills provided), to provide new prescriptions for corresponding medication, and to check the patients had not changed their lifestyles or had been prescribed additional pharmacological treatment that might affect their lipid or inflammatory status or oxidative stress levels.

At the end of the intervention period all patient variables (see above) were rechecked in all 3 study groups. All patients received strict clinical monitoring, with particular attention paid to liver enzymes levels.

The main adverse effects checked for were gastric intolerance of the drugs provided and/or liver enzyme levels three times the normal laboratory-reported limits. All patients were fully informed about the study and provided their written consent to be included; all were allowed to abandon the study at any time. This work was approved by the Ethics Committee of the Hospital General Regional Nº. 1 del Instituto Mexicano del Seguro Social in Morelia, Michoacán, México.

Blood Tests

Blood was collected between 7.00 and 8.00 am after a 12 h fast and with the patients having rested for 20 min. All samples were collected by trained personnel. The samples were then centrifuged at 4000 rpm for 15 min to extract the serum. Aliquots were prepared for the determination of glucose, TC, LDL-C, HDL-C, and TG by enzyme colorimetry using the Dimension® AR Clinical Chemistry System. The remaining aliquots were stored at -70ºC until they were analyzed for IL-6, TNFα, GSH, GPx, and SOD by ELISA (Cayman Chemical®). The intra-analysis coefficient of variation for all tests was 3%-5%.

Statistical Analysis

The results are expressed as means (standard deviation). The Student t test for paired samples was used to examine the differences in serum lipids before and after the pharmacological interventions. Differences between means were analyzed by 2-way ANOVA followed by the Bonferroni test. The dependent variables were the concentrations of IL-6, TNFα, and oxidative stress enzymes; the different treatments and times (before and after treatment) were taken as independent variables.

TABLE 1. **Baseline Clinical and Biochemical Characteristics of the Patients**

Variables	GRos (n=16)	GMetf (n=16)	GC (n=16)	P (ANOVA)
Age, mean (SD), y	56 (8.8)	52.25 (10.87)	54 (8.01)	.538
Body weight, kg	80.57 (12.83)	80.97 (10.22)	77.63 (11.11)	.739
Height, m	1.54 (0.09)	1.53 (0.05)	1.59 (0.06)	.096
BMI	33.05 (4.09)	34.39 (3.83)	31.02 (3.56)	.118
YWHBP	8.68 (7.57)	10.54 (4.67)	5.38 (4.88)	.194
SBP, mm Hg	132.68 (21.7)	142.06 (29.84)	132.12 (11.32)	.490
DBP, mm Hg	86.93 (10.19)	88.06 (12.69)	80.92 (15.23)	.785
Glucose, mg/dL	119.68 (32)	132.71 (48.82)	149 (66.37)	.309
TC, mg/dL	228.18 (25.51)	225.92 (12.71)	241.58 (33.4)	.253
LDL-C, mg/dL	130.29 (25.84)	129.91 (7.53)	130.43 (13.87)	.998
HDL-C, mg/dL	45.12 (11.73)	42.91 (4.31)	37.56 (19.85)	.440
TG, mg/dL	208.21 (74.16)	178.83 (38.54)	240.81 (81.79)	.104

YWHBP indicates years with high blood pressure; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; GC, control group; GMetf, metformin group; GRos, rosuvastatin group; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; TG, triglycerides.

TABLE 2. **Markers of Inflammation and Concentration of Oxidative Stress Enzymes at the Beginning of Treatment**

Variable	GRos (n=16)	GMetf (n=16)	GC (n=16)	P (ANOVA)
IL-6, pg/mL	12.45 (1.66)	13.39 (3.32)	14.52 (3.63)	.191
TNFα, pg/mL	8.74 (1.27)	8.66 (1.57)	8.36 (1.64)	.856
GSH, nmol/min/mL	10.42 (4.58)	10.06 (8.38)	13.42 (4.13)	.320
GPx, nmol/min/mL	18.31 (6.8)	14.2 (6.18)	15.77 (2.97)	.148
SOD, U/mL	0.3539 (0.05)	0.3526 (0.07)	0.3673 (0.05)	.769

A P value less than .05 was considered significant. All calculations were performed using SPSS v.12.0 software for Windows (Chicago, Illinois, USA).

RESULTS

No patients were lost to follow-up nor was there any need to suspend treatment in any patient during the 12 week experimental period. Treatment was well tolerated, no patient declared any adverse effect, and no significant modifications in liver enzyme values were seen. Tables 1 and 2 show the clinical, biochemical and inflammation, and oxidative stress marker results for the patients at the start of the study. The values of all variables across the groups were similar at this time.

A post-treatment reduction in body weight was seen in the GRos (80.57 [12.83] kg before treatment, 79.27 [12.52] kg after treatment; P=.013) and GMetf subjects (before treatment 80.97 [10.22] kg, after treatment 74.7 [10.44] kg; P=.011), and therefore in their BMI (GRos before treatment 33.05 [4.09] kg, after treatment 33.37 [3.62] kg [P=.002]; GMetf before treatment 34.39 [3.83] kg, after treatment 32.41 [4.79] kg [P=.015]). No significant changes in body weight nor BMI were seen in the GC subjects.

Figure 2 shows the percentage modification of the serum lipid profiles with respect to each treatment group.

In the GRos group, treatment reduced the TC by 41.7%, LDL-C by 63%, and TG by 10.7%, and increased HDL-C by 6.3%. In contrast, in the GMetf group there was a general trend towards an increase in serum lipids, especially LDL-C which showed an 11.8% increase.

Figure 3 shows the effect of the different treatments in terms of serum IL-6 and TNFα concentration. In the GRos group, IL-6 was reduced by 22.24% and TNFα by 13.03%; in the GMetf group IL-6 was reduced by 26.73% and TNFα by 8.31% (P<.05 for all comparisons). Two-way ANOVA revealed an interaction between the groups with respect to IL-6 (F=3.19; P=.045) and TNFα (F=8.01; P=.004), and significant differences between the groups GRos and GMetf compared to GP after 3 months with respect to IL-6 (F=12.50; P<.0001) and TNFα (F=3.12; P=.048).
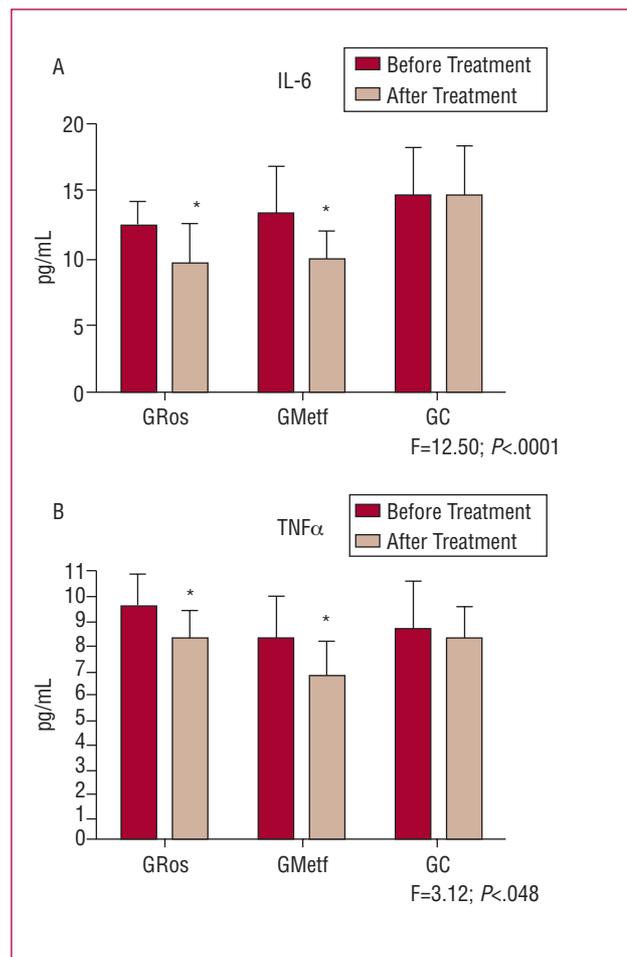
Finally, Figure 4 shows the change in oxidative stress markers for each group. The activities of GSH and GPx were both significantly reduced and SOD activity significantly increased by the GRos and GMetf treatments. Two-way ANOVA revealed an interaction between the groups with respect to GSH (F=4.46; P=.014), GPx (F=8.04; P=.0006), SOD (F=5.56; P=.008) and significant differences between the groups GRos and GMetf and GP after 3 months of treatment with respect to the same oxidative stress enzymes (GSH, F=17.74; P<.0001; GPx, F=11.38; P<.0001; SOD, F=9.11; P=.0004).
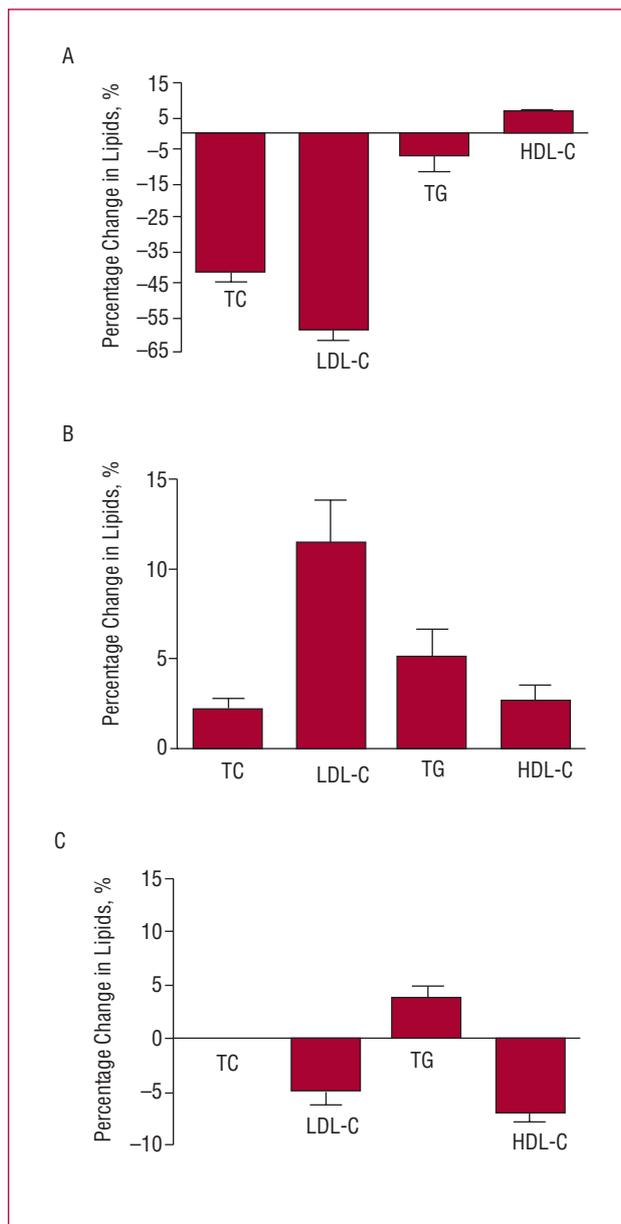
Figure 2. Percentage modification of lipids after 12 weeks of treatment. A: rosuvastatin group. B: metformin group. C: control group. HDL-C indicates high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

Figure 3. Modification of inflammation following 12 weeks of treatment. Two-way ANOVA (differences between GRos and GMetf compared to GC). GC indicates control group; GMetf, metformin group; GRos, rosuvastatin group.

DISCUSSION

Treatment with oral rosuvastatin (10 mg/day) for 3 months reduced the patients' TC, TG, and LDL-C levels, moderately increased the HDL-C level, and reduced the levels of inflammation and oxidative stress markers. Treatment with oral metformin (1700 mg/day) had a similar effect on the latter variables, but induced non-significant increases in lipid profile variables, especially LDL-C.

Statins (inhibitors of HMG-CoA reductase) can induce large reductions in the concentration of plasma lipids; they are therefore the treatment of choice for patients with hypercholesterolemia or high LDL-C concentrations. In the present study, significant reductions were seen in both TC and LDL-C concentrations following treatment with rosuvastatin (10 mg/day) However, it should be noted that this response was seen with a dose of just (10 mg/day); in other studies[18,19] such a response has only been seen with larger doses, which can be associated with more intense adverse effects. In the present work no patient reported any adverse event attributable to rosuvastatin, nor were any changes seen in the liver enzymes that might indicate a modification of hepatic function. The mechanism of action of this drug and of the statins in general involves the reduction of TC and LDL-C via the inhibition of hepatic cholesterol synthesis, and by increasing the expression of liver LDL-C receptors that favor the capture of this compound.
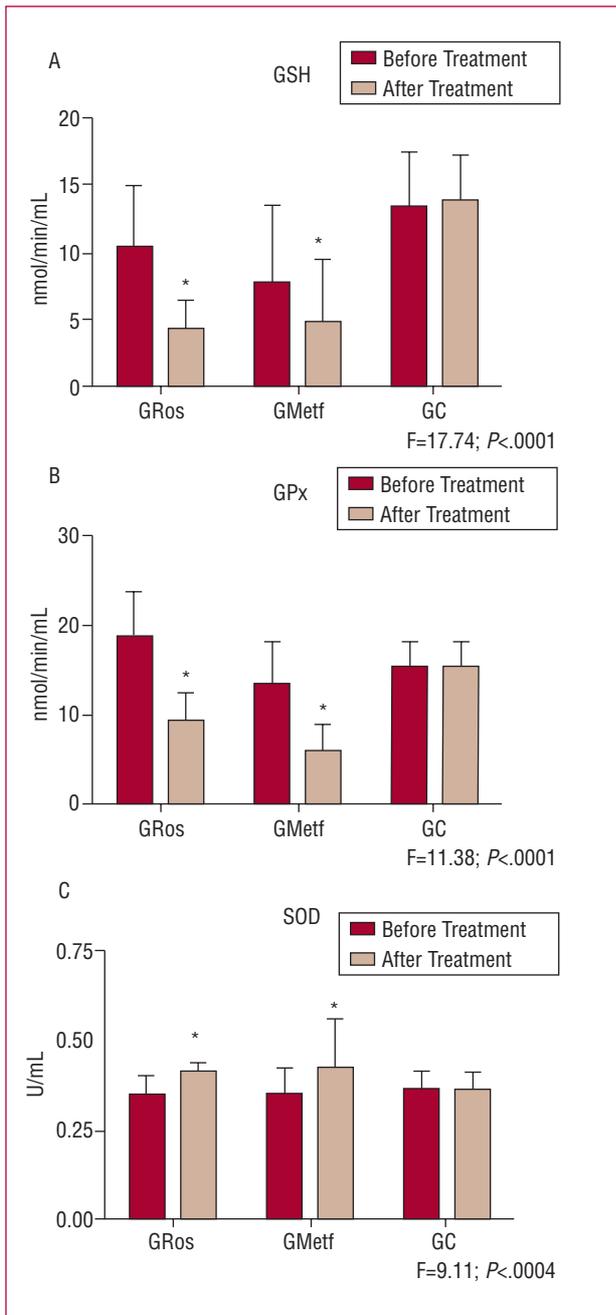
Figure 4. Modification of markers of oxidative stress after 12 weeks of treatment. Two-way ANOVA (differences between GRos and GMetf compared to GC). GC indicates control group; GMetf, metformin group; GRos, rosuvastatin group.

An interesting finding was the moderate loss of body weight (2.8 kg) associated with rosuvastatin treatment. This is thought to be the first report associating statin treatment with such weight loss. It may be that by reducing the serum lipid concentration sensitivity to insulin is improved. In patients with HBP and dyslipidemia it is common that a reduction in insulin resistance be accompanied by weight loss.[20] This hypothesis may receive some support from the reductions observed in serum IL-6 and TNFα, cytokines related to inflammation, and insulin resistance.[21]

Although it has been reported that metformin can reduce plasma lipid values,[22-24] in the present study no significant differences in serum lipid values were seen in the group treated with this drug. In agreement, Kiayias et al[25] reported metformin to have no effect on plasma lipid levels. The main metabolic effect of metformin is the improvement in sensitivity to insulin of the liver and peripheral tissues. The beneficial effect of metformin in terms of the reduction of body weight and of pro-insulin-like molecules has been reported.[26,27] In the present study, treatment with metformin 1700 mg/day led to a significant reduction in BMI; this agrees with that reported in other clinical studies[28,29] and confirms that previously reported by our group[30] - that the most important effects of metformin are weight loss, the modification of body composition, an increase in glucose uptake in hypoglycemic patients, and hyperinsulinemia and the improvement of beta cell function. Several authors have shown metformin eliminates plasminogen activator inhibitor 1 and macrophage migratory inhibition factor from the plasma of obese patients; this drug may therefore have anti-inflammatory activity and reduce cardiovascular morbidity/mortality.[31,32]

High blood pressure is reported to promote the endothelial expression of cytokines such as IL-6 and TNFα, which mediate the amplification of proinflammatory signals[33] and participate in the development of atherosclerosis.[34,35] There is therefore growing interest in the pleiotropic effects of drugs such as the statins and[36-38] and metformin,[39] which might help modulate oxidative stress and the inflammatory response (known cardiovascular risk factors). In the present work, the administration of rosuvastatin or metformin significantly reduced serum IL-6 and TNFα concentrations. The reduction of these inflammation markers is probably due to a reduction in the activity of nuclear factor kappa B (NF-κB) and an increase in the activity of the protein Akt (as seen in monocyte cultures).[39-41] Evidence has accumulated in recent years that NF-κB is a common denominator in the coordinated expression of genes induced by inflammatory processes associated with endothelial activation.[42] Unlike other transcription factors, the activation of NF-κB requires no induction of gene expression.

It is known that in patients with HBP, hyperglycemia, and dyslipidemia increase oxidative stress. In the present study, treatment with rosuvastatin or metformin led to a reduction of this stress. This might be explained by a direct effect of these drugs on the suppression of NF-κB, thus reducing inflammation and the production of reactive oxygen species,[41,43,44] or by their regulating the activity of SOD, which would help protect against oxidative stress.[45]

Limitations of the Study

This study has several limitations. For example, body composition was not measured by bioimpedance; therefore while the results indicate that rosuvastatin and metformin have a beneficial effect on body weight, it is not certain that this is due to the loss of fat. In addition, serum insulin concentrations were not recorded – this hormone has a known anti-inflammatory effect.[46] Clinical studies are needed to investigate the effects of insulin resistance in the peripheral tissues plus the interaction of different anti-hypertension drugs on oxidative stress.

CONCLUSIONS

The present results show that patients with HBP and dyslipidemia who are treated with rosuvastatin 10 mg/day experience a significant reduction in their serum TC, LDL-C, and TG concentrations, plus a moderate increase in their HDL-C concentration. Rosuvastatin and metformin significantly reduce inflammation and oxidative stress, and may therefore offer a protective effect against cardiovascular disease. Some of their pleiotropic effects are thus made manifest in the present results. Long-term clinical trials are needed to determine whether rosuvastatin and metformin can continue to reduce the cardiovascular risk caused by oxidative stress and inflammation in this type of patient.

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