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# Simvastatin treatment prevents oxidative damage to DNA in whole blood leukocytes of dyslipidemic type 2 diabetic patients

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Type 2 diabetes (T2D) is associated with increased oxidative stress as indicated by elevated levels of lipid peroxidation and protein oxidation products. Since reactive oxygen species (ROS) can cause damage to biological macromolecules including DNA, this study investigated oxidative damage to DNA using the alkaline (pH > 13) comet assay in peripheral whole blood leukocytes sampled from 15 dyslipidemic T2D patients treated with simvastatin (20 mg/day), 15 dyslipidemic T2D patients not treated with simvastatin, 20 non-dyslipidemic T2D patients, and 20 healthy individuals (controls). Our results showed a greater DNA migration in terms of damage index (DI) ($p < 0.01$) in the dyslipidemic T2D patients not treated with statin (DI = 67.70 ± 10.89) when compared to the dyslipidemic T2D patients under statin treatment (DI = 47.56 ± 7.02), non-dyslipidemic T2D patients (DI = 52.25 ± 9.14), and controls (DI = 13.20 ± 6.40). Plasma malondialdehyde (MDA) and C-reactive protein (CRP) levels were also increased and total antioxidant reactivity (TAR) and paraoxonase activity (PON1) decreased in non-dyslipidemic T2D patients and dyslipidemic T2D non-treated with simvastatin. We also found that DI was inversely correlated with TAR ($r = -0.61$, $p < 0.05$) and PON1 ($r = -0.67$, $p < 0.01$). In addition, there was a significant positive correlation between DI and CRP ($r = 0.80$, $p < 0.01$). Our results therefore indicate that simvastatin treatment plays a protective role on oxidative damage to DNA in dyslipidemic T2D patients probably reflecting a general decrease in oxidative stress in these patients. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS — comet assay; statins; dyslipidemic type 2 diabetes; paraoxonase; C-reactive protein

## INTRODUCTION

Type 2 diabetes mellitus (T2D) is one of the most common metabolic diseases, affecting about 3% of the human population.[1] Clinically, it is a heterogeneous disease, characterized by metabolic disorders, defective blood glucose control and is frequently associated with chronic complications such as retinopathy, nephropathy, and cardiovascular diseases.[2] Dyslipidemia is frequently associated with diabetes and cardiovascular disease.[3] The oxidative modification of low-density lipoprotein (LDL) plays a central role in the initiation and acceleration of atherosclerosis.[4,5] Diabetes mellitus is associated with increased oxidative stress as indicated by elevated levels of lipid peroxidation[6] and protein oxidation products.[7] Mechanisms that contribute to the formation of free radicals in diabetics may include non-enzymic and auto-oxidative glycation, increases in the levels of inflammatory mediators, metabolic stress resulting from changes in energy metabolism and status of antioxidant defense.[6] Under conditions of oxidative stress damage to cellular biomolecules such as lipids, proteins, and DNA occurs.

Oxygen free radicals induce a variety of lesions in DNA, including oxidized bases, abasic sites, DNA strand breaks, and cross-links between DNA and proteins. It has been shown that hydroxyl radical which is produced by the Fenton reaction in the presence of transition metal ions is responsible for DNA damage.[8] Direct interaction of the free radicals with DNA has been shown to cause genetic damage and disturbance of cell signaling in human cancers.[9]

Both increased oxidative stress and decreased antioxidant capacity can promote lipid peroxidation. The inverse correlation between high-density lipoprotein (HDL) cholesterol and risk of cardiovascular disease is well established, and a critical function of HDL is to mediate

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the efflux of cholesterol from vascular macrophages and other peripheral tissues.[10,11] In addition to the role in reverse cholesterol transport, HDL also has antioxidant, anti-inflammatory, and anti-thrombotic properties.[12–14]

Human serum paraoxonase 1 (PON1), which is an esterase associated with HDL,[15] reduces the susceptibility of LDL to lipid peroxidation *in vitro*.[16] Therefore, this enzyme is thought to play a central role in the inhibitory effect of HDL on lipid peroxidation. In fact, PON1 knockout mice have been shown to be susceptible to the development of atherosclerosis.[17–19] Several previous studies have shown that enzymatic activities of HDL-associated PON1 were decreased in patients with type 1 diabetes mellitus[15,20,21] and in those with T2D[20,22] as well as in streptozotocin-induced-diabetic rats.[23]

Statins have been largely used to treat hypercholesterolemia. The efficacy of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) inhibitors in lowering serum cholesterol concentrations is well known and has been extensively documented.[24] Many studies have shown that statins have several properties, including a potent antioxidant effect.[24,25]

The major aim of this study was to evaluate the levels of oxidative damage to DNA in the peripheral whole blood of non-dyslipidemic T2D patients, dyslipidemic T2D patients treated or not with simvastatin, and healthy age-matched control subjects. We also investigated whether simvastatin treatment could prevent genomic instability and the pro-oxidant status present in dyslipidemic diabetic patients.

## MATERIALS AND METHODS

### *Patients and blood sampling*

A total of 20 non-dyslipidemic T2D patients (8 males and 12 females; age range: 41–63 years), 20 dyslipidemic T2D patients not treated with statin (9 males and 11 females; age range: 42–76 years), 15 dyslipidemic T2D patients under statin treatment, simvastatin 20 mg/day (6 males and 9 females; age range: 45–72 years), and 20 healthy controls (8 males and 12 females; age range: 39–63 years) were studied. Average period of simvastatin treatment was 2 years. T2D patients were diagnosed and classified according to the American Diabetes Association criteria (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). All diabetic patients with moderate to severe hyperglycemia were under treatment with hypoglycemic agent (metformin 850 mg/day) and the mean time of treatment was 3 years. Dyslipidemia was considered with the combined presence of high triglyceride ($\geq$150 mg/dl), total cholesterol ($\geq$190 mg/dl), LDL cholesterol ($\geq$100 mg/dl), and low HDL cholesterol (<45 mg/dl). The control subjects consisted of healthy volunteers paired for age. Control subjects with known metabolic and/or infection disease were excluded from the study. Informed consent was obtained from all individuals after the purpose and nature of the study had been explained and this study was approved by the Ethics Committee of Clinical Hospital of Porto Alegre (HCPA). The samples were obtained at the Laboratory of Clinical Analysis of Pharmacy Faculty of Federal University of Rio Grande do Sul (UFRGS). Lifestyle habits and previous medical records were collected from each subject.

Blood samples were collected without and with EDTA after 12 h fasting. Plasma was removed by centrifugation and used for malondialdehyde (MDA), total antioxidant reactivity (TAR), C-reactive protein (CRP), paraoxonase activity (PON1), and protein determination. Comet assay and glycated-hemoglobin ($HbA_{1c}$) were determined in EDTA whole blood by electrophoresis and high performance liquid phase chromatography (HPLC), respectively. Serum was used for glucose and cholesterol measurements (Labtest® kits, Lagoa Santa, Minas Gerais, Brazil). All biochemical assays were carried out in duplicate or triplicate, depending on sample availability.

### *Reagents*

All chemicals used in this study were from Sigma® (St. Louis, MO, USA) and were of analytical grade.

### *Malondialdehyde determination (MDA)*

MDA was determined by HPLC following the method described by Karatepe.[26] One hundred microlitre of 0.1 M perchloric acid and 1 ml of distilled water were added to a 100 μl aliquot portion of plasma. Addition of acid was necessary to precipitate proteins and release the MDA bound to the amino groups of proteins and other amino compounds. The samples were centrifugated at 4500 rpm for 5 min and used for HPLC analysis. The mobile phase was 82.5:17.5 (v/v) 30 mM monobasic potassium phosphate (pH 3.6)-methanol and the flow rate was 1.2 ml/min. A reverse phase column (C18) was used and the chromatograms were monitored at 250 nm. The concentrations were calculated following external standardization. The calibration curve was drawn from a stock solution of 10 mM MDA.

### *Total antioxidant reactivity (TAR)*

TAR, which represents the total functional antioxidant capacity of a tissue, was determined by measuring the luminal chemiluminescence intensity induced by 2,2'-azo-bis-(2-amidinopropane) (ABAP) according to the method of Lissi *et al.*[27] The background chemiluminescence was measured by adding 4 ml of 2 mM ABAP (in 0.1 M glycine buffer, pH 8.6) into a glass scintillation vial. Fifteen microliters of luminol (4 mM) was added to each vial and the chemiluminescence was measured. This was considered to be the basal value. Ten microliters of 25–200 μM Trolox (curve calibration) or plasma was then added and the chemiluminescence was measured during 60 s. The Trolox or biological sample addition reduced the chemiluminescence. The rapid reduction in luminol intensity is considered as a measure of the TAR capacity. TAR measurement was calculated as nmol Trolox equivalents/mg protein.

 

*C-reactive protein concentration (CRP)*

CRP concentrations were determined by immunoturbidometry with Labtest® Kit (Lagoa Santa, Minas Gerais, Brazil).

*Single cell gel electrophoresis (comet assay)*

The alkaline comet assay was performed as described by Singh *et al.*,[28] in accordance with general guidelines for use of the comet assay.[29–31] Isolated human leukocytes were suspended in agarose and spread into a glass microscope slide pre-coated with agarose. Agarose was allowed to set at 4∘C for 5min. Slides were incubated in ice-cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10.0, and 1% triton X-100 with 10% DMSO) to remove cell proteins, leaving DNA as 'nucleoids'. After the lysis procedure, slides were placed on a horizontal electrophoresis unit, covered with a fresh solution (300 mM NaOH and 1 mM EDTA, pH > 13) for 20 min at 4°C to allow DNA unwinding and the expression of alkali-labilesites. Electrophoresis was performed for 20 min (25 V; 300 mA; 0.9 V/cm). Slides were then neutralized, washed in bi-distilled water and stained using a silver staining protocol.[32,33] After drying at room temperature overnight, gels were analyzed using an optical microscope. One hundred cells (50 cells from each of the two replicate slides) were selected and analyzed. Cells were visually scored according to tail length and receive scores from 0 (no migration) to 4 (maximal migration) according to tail intensity.[31] Therefore, the damage index (DI) for cells ranged from 0 (all cells with no migration) to 400 (all cells with maximal migration). The slides were analysed under blind conditions at least by two different individual median values of the scores were presented. We used readings mean of the scores which was agreement.

*Paraoxonase activity (PON1)*

Enzyme-activity was measured using paraoxon (Sigma®) as the substrate after the addition of plasma (50 μl) in Tris-HCl buffer (900 μl, 60 mM, pH 10.5 containing $CaCl_2$ 1.2 mM). The reaction was continuously monitored at 410 nm at 37°C. Results were expressed as IU. One unit of international enzyme activity is equal to 1 nmol of paraoxon hydrolysed per minute and per ml of plasma.[34]

*Protein determination*

Protein concentrations were determined by Biuret method (Labtest Kit®, Lagoa Santa, Minas Gerais, Brazil) using bovine serum albumin as standard.

*Statistical analysis*

All statistical analyses were performed using the SPSS software (15.0 version for Windows). Results of biochemical parameters are expressed as mean ± standard deviation (SD) and were analyzed by one-way analysis of variance (ANOVA) followed by Duncan multiple range test when $F$ value was significant. The Pearson and Spearman correlations were also used. Oxidative damage to DNA was analyzed by the Kruskal–Wallis test with the Mann–Whitney *post hoc* test. Values of $p < 0.05$ were considered statistically significant.

## RESULTS

Age and biochemical markers from controls and patients are shown in Table 1. Total cholesterol [$F(3.19) = 17.68$, $p < 0.01$] and LDL-cholesterol serum levels [$F(3.19) = 34.98$, $p < 0.01$] were significantly higher in dyslipidemic T2D patients not treated with simvastatin when compared with treated patients and control group. HDL-cholesterol serum levels [$F(2.49) = 11.5$, $p < 0.01$] were significantly lower in dyslipidemic T2D patients not treated with simvastatin when compared with treated patients and control group (Table 1).

Plasma markers of lipid peroxidation (MDA), TAR, PON1, and CRP from controls and patients are shown in Table 2. There was an increase in MDA [$F(3.28) = 46.13$, $p < 0.01$] and CRP [$F(3.38) = 33.16$, $p < 0.01$], as well as a decrease in TAR values [$F(3.21) = 5.6$, $p < 0.05$] and PON1 activity [$F(4.28) = 6.73$, $p < 0.05$] in non-dyslipidemic T2D

Table 1. Age and blood concentration of biochemical markers from patients and controls

| | Control | Non-dyslipidemic T2D patients | Dyslipidemic T2D patients not treated with simvastatin | Dyslipidemic T2D patients under simvastatin treatment |
|---|---|---|---|---|
| *N* | 18 | 11 | 14 | 9 |
| Age (years) | 49 ± 8.9 | 55 ± 6.6 | 57 ± 9.4 | 59 ± 8.7 |
| HbA1c (%) | – | 7.8 ± 2 | 7.5 ± 2 | 7.5 ± 1.4 |
| Glucose (mg/dl) | 93 ± 4.6[*,†] | 136 ± 47.9[‡] | 162 ± 69.1[‡] | 123 ± 27.8 |
| Total cholesterol (mg/dl) | 167 ± 25.4[†] | 179 ± 23.6[†] | 211 ± 43.1[*,‡,§] | 172. ± 39.8[†] |
| HDL-cholesterol (mg/dl) | 51 ± 10.8[†] | 47 ± 7.5[†,§] | 37 ± 5[*,‡,§] | 57 ± 9.5[*,†] |
| LDL-cholesterol (mg/dl) | 98 ± 11.2[†] | 100 ± 11.5[†] | 148 ± 23.5[*,‡,§] | 103 ± 16.7[†] |

Data represent mean ± SD and were analyzed by one-way ANOVA with the Duncan *post hoc* test ($p < 0.05$).
[*]Statistically different from non-dyslipidemic T2D patients.
[†]Statistically different from dyslipidemic T2D patients not treated with simvastatin.
[‡]Statistically different when compared to control.
[§]Statistically different when compared to dyslipidemic T2D patients treated with simvastatin.

 

Table 2. Plasma total antioxidant reactivity (TAR), malondialdehyde (MDA), paraoxonase activity (PON1), and C-reactive protein (CRP) in patients and controls

| | Control | Non-dyslipidemic T2D patients | Dyslipidemic T2D patients not treated with simvastatin | Dyslipidemic T2D patients under simvastatin treatment |
|---|---|---|---|---|
| TAR (nmolTrolox/mgprot) | $37 \pm 24$ | $24 \pm 10.6^{*}$ | $13 \pm 6.8^{*}$ | $56 \pm 22.2^{*, **}$ |
| MDA (μM) | $4.5 \pm 0.2$ | $5 \pm 0.2^{*}$ | $6 \pm 0.2^{*}$ | $4.3 \pm 0.2^{**}$ |
| PON1 (IU) | $341 \pm 80$ | $242 \pm 46^{*}$ | $169 \pm 41.7^{*}$ | $460 \pm 112^{**}$ |
| CRP (mg/L) | $3 \pm 1$ | $5 \pm 1.8^{*}$ | $6 \pm 2.8^{*}$ | $3 \pm 2^{**}$ |
| PON1/HDL ratio | $7 \pm 0.8$ | $5 \pm 0.3^{*}$ | $4 \pm 0.9^{*}$ | $8.2 \pm 2^{**}$ |
| PON1/CRP ratio | $122 \pm 6.1$ | $3.6 \pm 0.8^{*}$ | $2 \pm 0.5^{*}$ | $185 \pm 89^{**}$ |

Data represent mean ± SD.
$^{*}p < 0.05$ statistically different from control.
$^{**}p < 0.05$ statistically different from dyslipidemic T2D patients not treated with simvastatin. (One-way ANOVA with the Duncan *post hoc* test).

patients and dyslipidemic T2D patients not treated with simvastatin when compared to the control group. We can also observe in Table 2 that simvastatin revert these biochemical findings. In addition, there was an increase in paraoxonase activity/high density lipoprotein ratio (PON1/HDL) [$F(3.36) = 23.59$, $p < 0.01$] and PON1/CRP [$F(3.36) = 45.82$, $p < 0.01$] in T2D patients treated with simvastatin when compared to T2D patients not treated with simvastatin.

A greater degree of DNA migration ($p < 0.01$) was observed (Figure 1) in the dyslipidemic T2D patients not treated with simvastatin (DI = 67.7 ± 10.8) when compared to the dyslipidemic T2D patients treated with simvastatin (DI = 47.5 ± 7), non-dyslipidemic T2D patients (DI = 52.2 ± 9.1), and controls (DI = 13.2 ± 6.4). We also verified that DI was inversely correlated with TAR ($r = -0.6$, $p < 0.05$), PON1 ($r = -0.7$, $p < 0.01$), and HDL ($r = -0.6$, $p < 0.01$), PON1/HDL ratio ($r = -0.5$, $p < 0.01$), and PON1/CRP ratio ($r = -0.7$, $p < 0.01$) and there was a significant positive correlation between DI and CRP ($r = 0.8$, $p < 0.01$) and MDA ($r = 0.6$, $p < 0.01$) in dyslipidemic T2D patients treated or not with simvastatin. Besides, PON1 was inversely correlated with MDA ($r = -0.8$, $p < 0.01$) and CRP ($r = -0.9$, $p < 0.01$) and there was significant positive correlations between PON1 and TAR ($r = 0.6$, $p < 0.01$) and HDL ($r = 0.8$, $p < 0.01$) in dyslipidemic T2D patients treated or not with simvastatin.

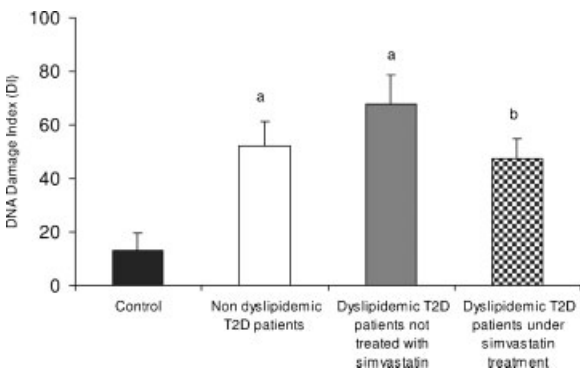


Figure 1. DNA damage index (comet assay) of peripheral whole blood leukocytes from patients ($n = 9$–14) and controls ($n = 18$). Data represent median ± SE (DI). (a) $p < 0.01$ when compared to treated patients and control. (b) $p < 0.01$ when compared to dyslipidemic T2D patients treated with simvastatin and non-dyslipidemic T2D patients and controls (Kruskal–Wallis test with the Mann–Whitney *post hoc* test)

## DISCUSSION

It is now recognized that reactive oxygen species (ROS) are involved in membrane peroxidation, DNA, and protein oxidation and inactivation of enzymes.[35,36] Moreover, it has been suggested that a chronic imbalance between formation of ROS and antioxidant capacity occurs in many pathological conditions, such as reperfusion injury, atherosclerosis, carcinogenesis, premature aging,[37,38] and also play an important role in the etiology and/or progression of diabetes.[39,40]

Hyperlipidemia, diabetes, hypertension, and smoking are well-established risk factors for the development of atherosclerosis. However, the molecular and cellular mechanisms linking these risk factors to a common pathologic mechanism are unclear. A current hypothesis suggests that modulation of the expression of a selective set of vascular inflammatory genes by intracellular oxidative signals may provide a molecular mechanism linking these diverse risk factors with the early pathogenesis of atherosclerosis.[41,42]

The efficacy of HMG-CoA reductase inhibitors (statins) in lowering serum cholesterol concentrations is well known and has been extensively documented.[43,44] The striking reduction in the incidence of ischemic cardiovascular events (stroke, acute myocardial infarction, and cardiovascular deaths) obtained with statins has led to the hypothesis that these drugs, besides lowering serum cholesterol, may act on the atherosclerotic plaque through alternative, ''stabilizing'' mechanisms involved in the disease progression.[43,45] Thus, it has been suggested that statins may induce activation of the nitric oxide (NO) pathway,[46] reduction in expression of adhesion molecules, and/or inhibition of proliferation and migration of vascular smooth muscle cells,[25] besides having anti-thrombotic[47] and anti-inflammatory responses.[48,49] Finally, statins have been reported to possess intrinsic antioxidant activity.[46,47] Franzoni *et al.*[43] measured the *in vitro* antioxidant capacity of statins as their ability to antagonize the oxidation of ∝-keto-γmethylbutyric acid by

 

both hydroxyl and peroxyl radicals and showed that all statins tested had significant antioxidant activity against these radicals. Fluvastatin, in particular, was the most active antioxidant toward peroxyl radicals, while simvastatin was the most active statin against hydroxyl radicals. Another study published by Aviram[50] has been proven that atorvastatin hydroxyl metabolites possess antioxidant potential, protecting LDL, VLDL, and HDL from oxidation induced by copper ion and other free-radical systems. This beneficial effect could be attributed mainly to the free-radical scavenging activity of atorvastatin metabolites.

In this study, we evaluated the levels of oxidative DNA damage in the leukocyte of non-dyslipidemic and dyslipidemic T2D patients treated or not with simvastatin and in healthy age-matched control subjects using the comet assay. We verified significantly greater levels of DNA migration, reflecting DNA damage, in dyslipidemic T2D patients not treated with simvastatin when compared to treated patients (simvastatin, 20 mg/day), non-dyslipidemic T2D patients and controls. In the alkaline (pH $> 13$) version of the comet assay developed by Singh *et al.*,[28] increased DNA migration can be associated with incomplete excision repair sites,[31] which are generated as an intermediate step during the action of different DNA repair systems.[51] When comparing the distribution of damage class in the studied groups, we observed that the difference was primarily caused by an increased number of cells in damage class 1, reflecting a homogeneously distributed increase in the number of slightly damaged cells rather than a low number of highly damaged cells. Our results are in accordance with other report. Shin *et al.*[52] showed that simvastatin (20–40 mg/day) administered for 8 weeks significantly reduced DNA damage of hypercholesterolemic patients. Studies published by Lodovici *et al.*[53] and Collins *et al.*[54] showed that patients with T2D patients have higher oxidative DNA damage than healthy subjects, which is in agreement with our data.

We also verified a significant increase in plasma MDA and CRP and a significant decrease in TAR and PON1 in non-dyslipidemic T2D patients and in dyslipidemic T2D patients not treated with simvastatin when compared to the control group. These data indicate induction of lipid peroxidation, an inflammatory response and a decrease in antioxidant defenses, all this representing important evidence of oxidative stress in these patients. Moreover, simvastatin treatment fully reverted these biochemical parameters, showing a protective effect against oxidative stress. We also found that DI was inversely correlated with TAR and PON1 and positively correlated with CRP and MDA in dyslipidemic T2D patients treated or not with simvastatin. These data strengthen the view that oxidative stress was mainly responsible for the DNA damage.

A significant increase of TBA-RS (lipid peroxidation) in plasma was observed in adult patients with T2D.[6] In fact, increased levels of the products of oxidative damage to lipids, proteins, and DNA have been detected in the serum of diabetic patients[6,55] and their presence was correlated with the development of diabetes complications.[56] Several clinical studies have also shown that the concentration of specific antioxidants in the plasma and erythrocytes of diabetic patients are reduced.[54,57]

TAR corresponds to a useful index of the capacity of a given tissue to modulate the damage associated with an increased production of free radicals and reflects the quality of antioxidants (given by its reactivity). In this study, DI was inversely correlated with TAR, reflecting that the decrease in tissue total antioxidants may have possibly led to greater oxidative damage to DNA.

Our data confirm the previous observations of low PON1 activity in T2D patients.[7,58] The low PON1 activity, which is important to hydrolyze lipid-peroxides, could therefore explain at least in part the increased lipid peroxidation commonly often reported in this disease.[59–61] In our study, we found that DI was inversely correlated with PON1 and with PON1/HDL ratio. In this context, biochemical studies indicate that PON1 is an HDL-associated esterase/lactonase and its activity is inversely related to the risk of cardiovascular diseases.[60] PON1 antiatherogenic properties include attenuation of oxidized-LDL uptake by macrophages, inhibition of macrophage cholesterol biosynthesis, and stimulation of HDL-mediated cholesterol efflux from macrophages.[50,56,62,63] Among the HDL subfractions, $HDL_3$, which is important to reverse cholesterol transport, carries the highest PON1 activity.[60]

Onat *et al.*[64] demonstrated that the CRP concentrations are increased in dyslipidemic patients secondary to the inflammatory process involved in this disease. Recent evidence indicates that statins decrease CRP levels in just 6 weeks of treatment, independent of LDL cholesterol reduction, and suggests that statins possess anti-inflammatory actions.[65,66]

Statins affect many of the events in the inflammatory cascade by inhibiting receptor-dependent activation of signal-transducing cascades. In a rat model of coronary inflammation, pravastatin reduced MCP-1 expression, monocyte infiltration, and proliferation.[25] Simvastatin reduced leukocyte rolling, adherence, and transmigration in a rodent model of NO deficiency and attenuated endothelial adhesion molecule[67] and monocyte CD11b expression[68] in the absence of lipid lowering. Statin therapy reduced the levels of soluble P-selectin in patients with acute coronary syndromes.[69] These observations underlie the importance of statins in attenuating the inflammatory process and the consequent impact on cardiovascular disease risk reduction. The positive correlation between CRP and DI verified in our dyslipidemic T2D patients as well as the negative correlation between DI and PON1/CRP may suggest that the inflammatory process involved in this disease may also contribute to oxidative damage to DNA.

Finally, statins are attractive therapeutic options for preserving normal vascular function and blood flow. In several human and animal studies, various statins have been shown to (1) inhibit the uptake and generation of ox-LDL;[70] (2) attenuate vascular and endothelial superoxide anion formation by inhibition of NADH oxidases via Rho-dependent mechanisms;[71] and (3) preserve the relative levels of vitamin E, vitamin C and endogenous antioxidants

 

such as ubiquinone and glutatione in LDL particles.[52,71,72] Thus, statins not only decrease oxidants but also restore antioxidants, thereby possibly reducing the level of oxidative stress in the vascular wall, which may explain some of the observed clinical beneficial effects.

In conclusion, our results may suggest that DNA injury in dyslipidemic T2D patients can be minimized with simvastatin treatment that seems to play a genoprotective role on the oxidative stress in these patients. Also we may suggest that PON1 and CRP concentration ratio could be used as a biochemical marker of oxidative and inflammatory damage in these patients.

## CONFLICT OF INTEREST

None known.

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