Effect of Simvastatin on Oxidative Stress Parameters and Lipid Profile in Type 2 Diabetic Patients

DR. NAJAH R. AL-MOUSAWI
DR. MOHAMMAD A. A. HUSSEIN
AMMAR RASOUL MOHAMMAD RUDHA

Abstract: Evidence has long existed regarding the relationship between oxidative stress & diabetes. The present study was conducted to assess the effect of simvastatin on selected oxidative stress parameters in form of (reduced glutathione (GSH), lipid peroxidation byproduct malondialdehyde (MDA) levels, glutathione –S- transferase (GST) activity & catalase (CAT) activity) & it is effect on lipid profile (total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) & very low density lipoprotein (VLDL) in dyslipidaemic type 2 diabetic patients. Fifty six dyslipidaemic type 2 diabetic patients were included in this study. A full history was taken & general examination was performed. Those patients were taking glipbenclamide (an oral hypoglycaemic drug) during the study as a treatment for their disease. The patients were followed up for 60 days & divided randomly into 2 groups. Group I (n = 31): no drug was given & served as dyslipidaemic diabetic control. Group II (n = 25): received simvastatin tablets 20 mg once daily at night. From those Fifty six patients included in this study, forty six patients reached the end of the study while ten patients withdrew (eight patients from Group I and two patients from Group II). This is due to non compliance of the patients. Blood samples were drawn from the patients at the beginning & after 60 days of follow up between 8:30 & 10:30 am after at least 1 hour fast. Fasting blood glucose, lipid profile, selected oxidative stress parameters (GSH, MDA levels, GST & CAT activities) were measured. Renal & hepatic functions were also assessed. This study revealed the following results: Simvastatin treatment increased serum GSH, reduced MDA levels significantly while did not significantly affect CAT & GST activity. In simvastatin treatment TC, TG, LDL & VLDL decreased significantly while HDL increased significantly. There were insignificant correlations between simvastatin induced changes in the oxidation markers & the observed changes of the lipid profile.
Introduction: Oxidative stress is defined as tissue injury resulting from a disturbance in the equilibrium between the production of reactive oxygen species (ROS) also known as free radicals and antioxidant defense mechanisms (1). Under physiologic conditions, the antioxidant defenses are able to protect against the deleterious effects of ROS, but under conditions where either an increase in oxidant generation, a decrease in antioxidant protection or a failure to repair oxidative damage, accumulation of free radicals ensures, leading to cellular & tissue damage (2). ROS are any molecular species capable of independent existence that contain one or more unpaired electrons in an atomic orbital (3). They include molecules like hydrogen peroxide, ions like hypochlorite ion, radicals like hydroxyl radical & superoxide anion which is both ion & radical (4). Excess generation of ROS in oxidative stress have pathological consequences including damage to polyunsaturated fatty acids in membrane lipids, proteins, DNA & ultimately cell death (5). ROS have been implicated in many disease state including neurodegenerative disease like Alzheimer’s & Parkinson’s disease, atherosclerosis, inflammatory conditions, certain cancers, diabetes mellitus (DM), cataract in the eye, pulmonary, renal, heart diseases & the process of aging (6,7). Diabetes mellitus is a group of metabolic disorders with one common manifestation: hyperglycaemia associated with defects in insulin secretion, action or both. Traditionally it has been classified into two forms Type 1 DM & Type 2 DM (8). Type 2 DM which is known to be multifactorial, resulting from combination of various factors such as impaired fatty acid metabolism, central fat deposition leading to insulin resistance in various tissues (liver, muscles, adipose) (9), beta-cell secretary defect & obesity (6). Evidence has long existed regarding the relationship between oxidative stress and DM (10). Eisei N. et al postulated that oxidative stress is involved in the onset and progression of diabetes, initiation and exacerbation of micro- and macrovascular complications in diabetes & recently oxidative stress status markers have been associated directly with the severity and prognosis of diabetes (11). There are multiple sources of oxidative stress in DM, including non enzymatic (glucose autoxidation, non enzymatic glycation of proteins), enzymatic (NADPH oxidase, nitric oxide synthase) & mitochondrial pathway (12). Dyslipidaemia is used to describe a group of conditions in which there are abnormal levels of lipid & lipoprotein in the blood (13). In type 2 diabetes, dyslipidaemia is characterized by elevated circulating levels of TG, decreased circulating levels of HDL & usually accompanied by an elevation of small dense LDL-cholesterol particles (14). There is an evidence indicating that hyperlipidaemia is associated with enhanced oxidative stress (15). Simvastatin belong to 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins which are potent inhibitors of cholesterol biosynthesis that are used extensively to treat patients with hypercholesterolaemia (16,17). Simvastatin is derived from a fermentation product of Aspergillus terreus via a semisynthetic processes. It is an inactive lactone prodrug & hydrolyzed to the corresponding β-hydroxyacid form (18). This is an inhibitor of HMG-CoA reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate which is an early & rate limiting step in the biosynthesis of cholesterol resulting in depletion the intracellular supply of cholesterol (19). Inhibition of cholesterol biosynthesis is accompanied by an increase in hepatic LDL receptor on the cell surface which promotes uptake & clearance of circulating LDL. Thus the end result is a reduction in plasma cholesterol both by lowered cholesterol synthesis & by increased catabolism of LDL (17). Simvastatin also reduce VLDL-C, TG & produce variable increase in HDL-C (20). Simvastatin is safe & generally well tolerated (18). Mild gastrointestinal side effects like dyspepsia, flatulence, abdominal pain, diarrhea & constipation. Others headache, rash, pruritus & malaise. The most detrimental adverse effects of simvastatin is hepatotoxicity & myopathy (21). Liao J K. & Shishehbor M H. et al stated that the overall clinical benefits observed with simvastatin therapy appear to be greater than what might be expected from changes in lipid levels alone, suggesting effects beyond cholesterol lowering.
called pleiotropic effects \(^{(17,22)}\). Vishal T. et al indicated that some of the cholesterol-independent effects of simvastatin involve improving endothelial function, enhancing the stability of atherosclerotic plaques, decreasing oxidative stress, decreasing inflammation, improve insulin resistance, inhibiting the thrombogenic response in the vascular wall & impede tumour cells. Further more statin have other extrahepatic beneficial effects on the immune system, central nervous system & bone \(^{(23)}\). Simvastatin possesses antioxidant properties by reducing lipid peroxidation & ROS production \(^{(23)}\). Simvastatin reduces the susceptibility of lipoproteins to oxidation both in vitro and in vivo i.e. they decrease the LDL oxidation \(^{(25)}\). Sobal G. et al observed that simvastatin besides its lipid lowering action had also significant antioxidative properties in diabetic patients \(^{(24)}\).

The Aim of This Study was to clarify the effect of simvastatin on selected oxidative stress parameters namely (reduced glutathione (GSH), lipid peroxidation product MDA levels, glutathione –S- transferase (GST) & catalase (CAT) activities) & lipid profile in dyslipidaemic type 2 diabetic patients.

Patients, Materials & Methods: Fifty six patients (age : 57.16 ± 1.34 years ; 30 men & 26 women) with type 2 DM (mean fasting blood glucose 7.91 ± 0.7 mmol / l, with a mean duration of diabetes of 8.4 ± 1.08 years) & dyslipidaemia (mean LDL-C level 5.48 ± 0.72 mmol / l) attending Al- Hakeem center for researches & treatment of DM in Al-Sadr Teaching Hospital in the period between 5 th Nov. 2006 to 24 th June 2007 were included in this study. These patients underwent full history & complete physical examination. Patients with the following criteria were excluded from the study : 1- Patients who use any vitamin preparation, or statins in the last three months \(^{(25)}\). 2- Patients with renal insufficiency, defined as a serum creatinine level equal to or more than 1.8 mg / dl \(^{(22)}\). 3- Patients with liver disease \(^{(22)}\). 4- Hypertensive patients, because this condition affects oxidative stress \(^{(15)}\). In addition to this, antihypertensive drugs may affect lipid profile & oxidative stress in hypertensive patients \(^{(26,27)}\). 6- Patients with chronic inflammatory diseases \(^{(25)}\). 7- Alcoholics & smokers were also excluded \(^{(28)}\). Those patients were taking glibenclamide (Glibesyn . Medochemie LTD-Cyprus, Glibils. Hikma-Jordon) (an oral hypoglycaemic agent) during the study as a treatment for their disease that is diabetes. According to the design of the study, type 2 diabetic patients were followed up for 60 days & divided randomly into two groups : 1- Group I (n = 31) : No drug was given & served as dyslipidaemic diabetic control. 2- Group II (n = 25) : Received Simvastatin tablets 20 mg once daily at night (Simvatin . Pharma International Co . Amman – Jordon . Batch no. 1769). From those Fifty six patients included in this study, forty six patients reached the end of the study while ten patients withdrew (eight patients from Group I and two patients from Group II). This is due to non compliance of the patients. The patients were put on diet control & followed every two weeks during the time of the study in order to make sure that they were using the medication properly, to supply the drug to the patients & to regularly check fasting blood glucose. Values of fasting blood glucose before, during & after the study were controlled within the previously mentioned range, they were comparable between the groups. Blood samples were drawn from the patients at the beginning & after 60 days of follow up between 8:30 & 10:30 am after at least 1 -1 hours fast. Fasting blood glucose, lipid profile, selected oxidative stress parameters (GSH, MDA levels, GST, CAT activities) were measured. Renal & hepatic functions were also assessed.

Serum GSH assay: Serum GSH was estimated according to a modified method utilizing Ellman reagent (DTNB) \(^{(29)}\). The used reagents were supplied by Biochemicals Co. Ltd for EDTA and GSH, Sigma Co. Ltd for DTNB. The assay mixture contained serum and DTNB 0.01 (5,5'-dithiobis-(2-nitrobenzoic acid), trichloroacetic acid (TCA 50 %), tris-EDTA buffer (0.2 M) PH 8.9, EDTA Na2 (0.2M) and GSH standards for preparation of stock standard solution &
standard calibration curve in µM (Figure-1). The net results read at 412 nm by using (Shimadzu UV-visible 1650 PC) spectrophotometer.

(15)


Figure-1: Standard curve for GSH determination

Serum MDA assay: The level of serum MDA was determined by a modified procedure described by (Guidet B. and Shah SV.) (30). All the chemicals were supplied by Merck Co. Ltd. The assay mixture contained serum and 17.5% TCA, 70% TCA, 0.6% thiobarbituric acid (TBA) and (Shimadzu UV-visible 1650PC) spectrophotometer used to read the final sample at 532 nm. The concentration of MDA was expressed in molar.

Serum GST enzyme activity assay: Serum GST activity determined by Habig WH. et al method (31). The used reagents were supplied by Analar grade for CDNB, K2HPO4 and KH2PO4. Biochemicals Co. Ltd for GSH. The assay mixture contained serum, GSH, 1-chloro,2,4-dinitrobenzene (CDNB), phosphate buffer (PH 6.25) and (Shimadzu UV-visible 1650PC) spectrophotometer used to read the final sample at 340 nm.

Serum catalase activity assay: Serum catalase activity determined by Aebi H. method (32). The used reagents were supplied by Analar grade for Na2HPO4, KH2PO4 and H2O2. The assay mixture contained diluted serum, phosphate buffer, hydrogen peroxide (30 mM) and (Shimadzu UV-visible 1650PC) spectrophotometer used to read the final sample at 240 nm.

Serum lipid profile assay: Total cholesterol, triglyceride, high density lipoprotein were measured according to procedures supplied by BioMerieux Company using Shimadzu UV-visible 1650PC spectrophotometer. Serum LDL & VLDL measured according to the Friedewald equation (33): VLDL = TG / 2.2.

LDL = TC – HDL – VLDL.

Statistical methods: The data expressed as mean ± SEM unless otherwise stated. Statistical analyses were done by using paired t-test. Pearson's correlations were also performed with significant difference was set at P < 0.05.

Results:

Effect of simvastatin on oxidative stress parameters: Simvastatin treatment increased serum GSH, reduced MDA level significantly while did not significantly affect serum GST & CAT
activity. Oxidative stress parameters insignificantly changed in diabetic control group apart from significant increase in MDA level (Table-1).

Table-1: Effect of simvastatin (20 mg / day) on oxidative stress parameters after 60 days of treatment & changes in dyslipidaemic diabetic control (n=23 in each group).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic control</th>
<th>Simvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>GSH (mmol/l)</td>
<td>0.24±0.0079</td>
<td>0.22±0.0036</td>
</tr>
<tr>
<td>MDA (mol/l)</td>
<td>1.25×10^{-4}±0.0146</td>
<td>1.59×10^{-4}±0.0210</td>
</tr>
<tr>
<td>GST (U/l)</td>
<td>13.68±0.18</td>
<td>13.87±0.2194</td>
</tr>
<tr>
<td>CAT (K/ml)</td>
<td>0.49±0.0123</td>
<td>0.501±0.016</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM.

Effect of simvastatin on lipid profile: Simvastatin treatment decreased serum level of TC, TG, LDL and VLDL significantly while significantly increased HDL level. Lipid profile did not significantly change in diabetic control group (Table-2).

Table-2: Effect of simvastatin (20 mg / day) on lipid profile after 60 days of treatment & changes in dyslipidaemic diabetic control (n=23 in each group).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic control</th>
<th>Simvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>6.99±0.1173</td>
<td>6.74±0.0332</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>2.78±0.0645</td>
<td>2.68±0.0159</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>0.81±0.0300</td>
<td>0.76±0.0114</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>4.90±0.1300</td>
<td>4.75±0.0339</td>
</tr>
<tr>
<td>VLDL (mmol/l)</td>
<td>1.26±0.0129</td>
<td>1.21±0.0031</td>
</tr>
</tbody>
</table>
Values expressed as mean ± SEM.
Correlations between observed changes in oxidation markers & observed changes in lipid parameters in simvastatin group: There were non significant correlations between simvastatin induced changes in the oxidation markers & the observed changes in the lipid profile (Table- 3).
Table- 3: Pearson's correlation for changes in the oxidative markers & lipid parameters in the simvastatin group.

<table>
<thead>
<tr>
<th></th>
<th>GSH</th>
<th>MDA</th>
<th>GST</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.063</td>
<td>0.059</td>
<td>0.323</td>
<td>0.135</td>
</tr>
<tr>
<td>TG</td>
<td>-0.077</td>
<td>-0.115</td>
<td>0.150</td>
<td>0.337</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.172</td>
<td>0.409</td>
<td>-0.025</td>
<td>0.095</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.145</td>
<td>-0.092</td>
<td>0.305</td>
<td>0.042</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>-0.110</td>
<td>-0.062</td>
<td>0.133</td>
<td>0.268</td>
</tr>
</tbody>
</table>

P > 0.05 non significant.

Discussion:
Effect on oxidative stress parameters: Serum GSH level increased significantly following simvastatin treatment and this finding was in agreement with that reported by Skrha J. et al (34). Also simvastatin showed a significant reduction in the MDA level that is the same result reached by Broncel M. et al (35). The increment of GSH & reduction of MDA by simvastatin in our study was attributed to the antioxidant mediated effect of simvastatin which result from inhibition of mevalonate pathway leading to the reduction in the synthesis of important intermediates including isoprenoids (farnesyl pyrophosphate & geranylgeranyl pyrophosphate) which serve as lipid attachments for intracellular signaling molecules in particular inhibition of small GTPase binding proteins (Rho, Rac, Ras & G proteins) whose proper membrane localization & function are dependent on isoprenylation. These proteins modulate a variety of cellular processes including signaling, differentiation & proliferation (36,37). Serum GST enzyme activity did not significantly change in simvastatin treatment and this finding was consistent with Passi S. et al (38) who concluded that simvastatin had no effect on GST activity. Various studies revealed conflicting results regarding the effect of simvastatin on CAT activity. Ungureanu D. et al stated that simvastatin caused a significant reduction in the CAT activity (39) while Broncel M. et al said that CAT activity was significantly increased by the effect of simvastatin (35). In the present study simvastatin caused non significant change in the CAT activity that is the same finding obtained by Passi S. et al (38). This discrepancy was due to the fact that the sample size may be relatively small permitting chance observations to exert substantial effects.

Effect on lipid profile: Simvastatin treatment decreased serum level of TC,TG, LDL and VLDL significantly while increased serum HDL significantly and this finding was in agreement with that obtained by Recto C.S. (40) and Udawat H. et al (41). The mechanism involved was largely attributed to the ability of simvastatin to impair cholesterol synthesis via inhibiting the enzyme HMG-CoA reductase which is the rate limiting step in cholesterol biosynthesis. This both decreases circulating lipoproteins & increases their uptake by up regulating hepatic LDL-C receptors. The overall lipid lowering effect include increase uptake & degradation of LDL-C, inhibition of LDL-C oxidation, reduction in cholesterol accumulation & esterification & decreases lipoprotein secretion & cholesterol synthesis (42,22).
Correlations between the observed changes in the oxidation markers & the improvement of lipid profile in simvastatin: According to this study there were insignificant correlations between the observed changes in the pleiotropic effect of simvastatin regarding antioxidant properties & the improvement in the lipid profile, that is the same finding reached by Koh KK et al (43). This pleiotropic effect of simvastatin is due predominantly to inhibition of isoprenoids but not cholesterol synthesis (44).

References:


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