Original Research Article

Atorvastatin up-regulates the expression and activity of renal Cytochrome P450 3A2 in diabetic rats

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ABSTRACT

Effects of atorvastatin on the expression of Cytochrome P450 3A2 and its enzymatic activity in the kidney of diabetic rats were investigated. Diabetes was induced by injection of streptozotocin (50 mg/kg, b.w., i.p.) in male Wistar rats. The animals were assigned into four groups including control (C), non-treated diabetic (D), atorvastatin-treated diabetic (AD) and atorvastatin-treated non-diabetic (A) groups. Metabolism of testosterone was examined in the presence of renal microsomes. The expression of CYP 3A2 at mRNA level was examined by means of PCR technique. The atorvastatin administration resulted in a remarkable improvement of diabetes-induced nephropathy and oxidative stress. Enzyme kinetics analyses showed that both diabetic groups produced significantly ($P < 0.05$) more 6β-hydroxytestosterone (Vmax for D = 34.7±1.3 and for AD = 45.1±2.3 pM/min/mg) than that of the control group (Vmax = 21.6±1.5 pM/min/mg). Both diabetes and atorvastatin administration resulted in a significant up regulation of CYP 3A2 mRNA level in the kidney. Our data suggest that due to profound influence of diabetes and atorvastatin on renal metabolism of CYP 3A2 substrate and up-regulation of this gene, there should be adjusted dose regimen for medications which are classified as CYP 3A2 substrates.

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Introduction

Diabetes mellitus (DM) is major cause of nephropathy that associates with high morbidity and mortality due to accelerated cardiovascular diseases (Sarnak et al., 2003). Interaction between several environmental and genetic factors contributes in the development and progression of diabetic nephropathy (DN) (Murea et al., 2012). Diabetes-induced increase in the formation of advanced glycation end
products alters the function of proteins and results in histopathological injuries. Histological injuries in the kidneys include thickening of the glomerular basement membrane, increased fractional mesangial volume, and podocyte abnormalities (Zelmanovitz et al., 2009).

The kidneys along with other organs including the liver and intestinal serve as drug and xenobiotics biotransforming tissues. In the kidneys of mammalian like liver, due to the heterogeneity of tissue, enzyme systems distributed on a cellular and subcellular levels (Lohr, 1998). Cytochrome P450 (CYP 450) – dependent mixed function oxidase system is the most well-known drug metabolism system in the kidneys, which mainly catalyzes the hydroxylation of various indigenous and exogenous compounds. By using molecular biology techniques, various isozymes of CYP 450 have been isolated from different species of mammalian including humans. There are reports indicating that rat CYP3A2 exhibits a 73% homology of the amino acid sequence and also functional analogies to human CYP3A4, which is responsible for the oxidative metabolism of majority of currently used drugs (Soucek and Gut, 1992; Gibson et al., 2002). There is evidence indicating the localization of microsomal CYP 450 in various regions with dominant localization in the cortex area of the kidneys (Hu et al., 1993).

Epidemiological evidence is suggesting that diabetes is a leading cause of chronic kidney disease (CKD), which characterized by proteinuria and renal insufficiency (Tonelli et al., 2012). At the same time both diabetes and CKD are considered as major risk factors of coronary heart disease, suggesting prioritizing of cardiovascular disease-related factors such as dyslipidemia in diabetic patients (Sarnak et al., 2003). Previously, an increase in low density lipoprotein cholesterol (LDL-C) has been recognized as a strong predictor of heart disease in diabetic patients (Li et al., 2013). For this reason there are treatment recommendations which offer LDL-C lowering with statins in addition of lifestyle changes in diabetic individuals. Statins are the HMG-CoA reductase inhibitors that reduce intracellular cholesterol synthesis via inhibition of l-mevalonic acid production by up-regulating of the expression of hepatic LDL receptors and also increasing the clearance of LDL-cholesterol from the circulation. Although statins have been known as well tolerated drugs with excellent safety, there are however lack of knowledge about the effect(s) of statins on drug/xenobiotic metabolism system including CYP 450. The aim of the present study was to evaluate the effect of atorvastatin on renal metabolism of testosterone as an endogenous compound by isolated microsomes from diabetic animals. Moreover, the expression of CYP 3A2 as major isozyme in adult male rats also was evaluated. Ultimately, the possible therapeutic effects of atorvastatin on diabetes-induced oxidative stress, histopathological injuries and renal functions as biomedical parameters were tested.

Materials and methods

Chemicals

Atorvastatin (A, PZ 0001), streptozotocine (STZ, S 0130), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), testosterone (T6147), 6β-hydroxy testosterone (H2898), and 11β-hydroxytestosterone (H4128) were purchased from Sigma-Aldrich (Germany). Thiobarbituric acid, phosphoric acid (85%), and dimethyl sulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany). N-butanol was obtained from Carl Roth, GmbH Co. (Germany). TRI reagent was purchased from Applied Biosystems, by life technologies (Nieuwerkerk, The Netherlands). Commercially available standard kits were used for the determination of blood urea nitrogen (BUN, Ziest Chem Diagnostics, 10-503, Tehran, Iran) and creatinine (PARS AZMUN, 90008, 01-400 009, Tehran, Iran). All other chemicals were commercial products of analytical grade.

Animals and experimental design

Thirty two adult healthy male Wistar rats (200–225 g) were obtained from the animal house of the Faculty of Veterinary Medicine, Urmia University. The animals were acclimatized for one week and had free access to food and water. The experimental protocols were approved by the ethical committee of Urmia University in accordance with the principles of laboratory animal care (NIH publication no. 85–23, revised 1985). The animals were assigned into four groups including control (C), non-treated diabetic (D), atorvastatin-treated diabetic (AD) and atorvastatin-treated non-diabetic (A) groups (n = 8). Atorvastatin was administered one week after diabetes-induction and for two weeks to mimic the situation of patients, which have the diabetes-related complications including renal associated disorders (proteinuria and polyuria). The dose levels for atorvastatin (10 mg/kg, orally) was selected based on previously published studies (Lau et al., 2006). The control and non-treated diabetic groups received only vehicle during the experiment. Before and after the treatment period, all animals were weighed individually and this procedure was repeated at the end of study to evaluate any treatment-related changes in the body weight gain.

Diabetes induction

To induce diabetes, the rats in the diabetic groups were injected intraperitoneally (i.p.) with freshly prepared streptozotocine (STZ) in 0.01 M citrate buffer, pH 4.5, at a single dose of 50 mg/kg, b.w. The control animals received only the vehicle. Blood glucose level was determined 24, 48 and 72 h after STZ injection and once a week using a glucose monitor set (Elegance, CT-X10, Convergent Technologies GmbH & Co. KG, Marburg, Germany). The animals were considered diabetic if the blood glucose level was >250 mg/dl. Daily volume of urine and the urine protein concentration were measured on the last day of experiment to highlight both the diabetes effect and also the possible therapeutic effect of atorvastatin.

Collection of blood, tissue samples and serum preparation

Following diabetes-induction and 14 days atorvastatin administration, blood samples were collected directly from the heart of the animals after light anesthesia which provided by diethyl ethers. Serum samples were obtained by centrifugation of the blood samples at 3000 rpm for 5 min at room temperature and thereafter, the serum samples were stored at
-20 °C until analysis onset. After blood collection, the animals were euthanized using overdose injection of sodium pentobarbital (200 mg/kg, b.w., i.p.) and the kidney tissues were dissected immediately. The collected tissue samples were divided into two parts and the first part after washing with chilled saline, were snap frozen in liquid nitrogen and then immediately were stored at -70 °C for further biochemical and molecular analysis. The second part of the samples was preserved in 10% buffered formaldehyde for further histopathological examinations.

Renal microsomes isolation

The microsomal fractions of the kidney samples were isolated as described previously (Malekinejad et al., 2005). Briefly, immediately after euthanasia the kidney specimens were collected, cut into small pieces, and rinsed three times with chilled saline normal. Two volumes of potassium chloride (KCl; 1.15%) and ethylenediaminetetraacetic acid (EDTA; 0.1 mM) solution were added to tissue samples, and the mixtures were homogenized in a Potter–Elvehjem apparatus with a Teflon pestle. The homogenates were centrifuged at 9000 × g at 4 °C for 30 min, thereafter the supernatant was further centrifuged at 100,000 × g at 90 min in order to obtain the microsomal fraction. After centrifugation, the supernatant was discarded, and the pellet was carefully dissolved in the same volume of phosphate buffer (0.05 M, pH 7.4) with glycerol (20%) and then homogenized with an Ultra-Turrax. The microsomal fractions were stored at -70 °C until use. Protein concentrations of the microsomal fractions were determined according to Lowry method (Lowry et al., 1951).

Hydroxylation assay of testosterone in rat renal microsomes

Testosterone 6β-hydroxylase activity was determined as described previously (Paolini et al., 1996). Renal microsomal protein (250 μg) was incubated in total volume of 0.5 ml phosphate buffer (200 mM, pH 7.4) containing a NADPH-generating system (3 mM MgCl2, 12 mM glucose-6-phosphate, and 0.25 U of glucose-6-phosphate dehydrogenase). The reaction solution was preincubated for 2 min after the addition of various concentrations of testosterone (5, 10, 50, 100 and 200 μM), which was dissolved in dimethyl sulfoxide (final concentration of DMSO was 1%). The reaction was started by adding the NADPH-generating system. Incubations were conducted at 37 °C in a shaking water bath for 30 min. The concentration of 6β-hydroxy testosterone in the reaction mixture was measured by High-Performance Liquid Chromatography (HPLC) after extraction.

HPLC analyses

6β-Hydroxy testosterone concentration was determined using HPLC, according to previously described method (Paolini et al., 1996). The HPLC analysis was performed on an Agilent 1200 HPLC system (Milford, MA, USA) equipped with a Quart pump (G 1311 A), an auto-sampler (G 1329 A), and a UV detector (VWD, G 1314 B). Twenty microliters of the extracted sample was injected onto a LUNA 5 μ C18 (150 × 4.60 mm, Phenomenex) column. The mobile phase consisted of a mixture of solvent A [25% methanol and 75% H2O (v/v)] and solvent B [64% methanol, 6% acetonitrile and 30% H2O (v/v/v)] was eluted at a flow-rate of 0.8 ml/min.

6β-Hydroxy testosterone was separated by gradient, from 90% to 50% (v/v) of solvent A in 45 min. 6β-Hydroxy testosterone was detected at 254 nm by means of a UV detector (VWD, G 1314 B). The concentration of 6β-HT was determined by the ratio between peak areas of 6β-HT and internal standard (11β-HT); calculation was made from calibration curves obtained with pure synthetic testosterone metabolites at 5-point set (Sigma–Aldrich, Germany).

Biochemical analysis

Blood glucose concentration was measured before and after the treatment in both major groups of the study. The serum levels of BUN and Creatinine were determined by using BUN (Ziest Chem Diagnostics, 10-503) and creatinine (PARS AZMUN, 90008, 01-400 009, Tehran, Iran) kits and according to manufacturer’s instructions.

Total sulfhydryl level in the kidneys was measured as described previously (Ranjar et al., 2006). Briefly, 0.3-0.4 g of the kidney samples was homogenized in ice-cold KCL (150 mM), and the mixture was centrifuged at 3000 × g for 10 min. To 0.2 ml of the supernatant of tissue homogenate, 0.6 ml Tris-EDTA buffer (Tris base 0.25 M, ethylenediaminetetraacetic acid 20 mM, pH 8.2) and thereafter, 40 μl 5,5′-dithiobis-2-nitrobenzoic acid (10 mM in pure methanol) were added. The final volume of this mixture was made up to 4.0 ml by an extra addition of pure methanol. After 15 min incubation at room temperature, the samples were centrifuged at 3000 × g for 10 min and ultimately the absorbance of the supernatant was measured at 412 nm. The TTM capacity was expressed as nmol per mg of protein in samples. The protein content of the samples was measured according to the Lowry method (Lowry et al., 1951).

To determine the lipid peroxidation rate in the control and test groups, MDA content of the kidney samples was measured using the thiobarbituric acid (TBA) reaction as described previously (Niehaus and Samuelsson, 1968). Briefly, 0.2–0.3 g of the samples were homogenized in ice-cold KCl (150 mM), and then the mixture was centrifuged at 3000 × g for 10 min; 0.5 ml of the supernatant was mixed with 3 ml phosphoric acid (1% V/V) and then after vortex mixing, 1 ml of 6.7 g L-1 TBA was added to the samples. The samples were heated at 100 °C for 45 min, and then chilled in ice. After addition of 3 ml N-butanol, the samples were centrifuged at 3000 × g for 10 min. The absorbance of supernatant was measured spectrophotometrically (Pharmacia, Novaspec II, Biochrom, England), at 532 nm and the amount of lipid peroxidation end product was calculated according to the simultaneously prepared calibration curve by using MDA standards. The amount of MDA was expressed as nmol per mg of protein. The protein content of the samples was assessed based on Lowry method (Lowry et al., 1951).

The total nitrate/nitrite content of the kidney samples was measured according to the Griess reaction (Green et al., 1982). In Griess reaction nitric oxide rapidly converted into more stable nitrite, which in an acidic environment nitrite is
converted to HNO₂. In reaction with sulphanilamide, HNO₂ forms a diazonium salt, which reacts with N-(1-naphthyl) ethylenediamine 2HCl to form an azo dye that can be detected by absorbing at 540 nm wavelength. The NO content of the liver was expressed as nmol per mg of protein in samples.

**Histopathological examinations**

To show the diabetes-induced pathological alterations in the renal structure and possible therapeutic effect of the atorvastatin treatment, Hematoxyline & Eosin staining was performed on the kidney samples. To evaluate the pathological damages and also the atorvastatin treatment effects on renal structure, indices such as the number and size of dilated tubules in cortex and medulla of the kidney and also the dilation of urinary space by using a calibrated eyepiece were scored numerically. For each animal in the tests and control groups at least three slides from the kidney samples were prepared and the aforementioned indices were measured at least in 30–45 microscopic fields. The pathological examinations were performed by a pathologist who was completely unaware of the study purposes.

**RNA isolation and RT-PCR**

To evaluate the effects of diabetes and Atorvastatin administration on mRNA level of CYP3A2, total RNA was isolated from the kidney samples using the standard TRIZOL method. The RNA amount was determined spectrophotometrically and the samples were stored at −70 °C. For RT-PCR, cDNA was synthesized in a 20 μl reaction mixture containing 1 μg RNA, oligo(dT) primer (1 μl), 5× reaction buffer (4 μl), RNAse inhibitor (1 μl), 10 mM dNTP mix (2 μl) and M-MuLV Reverse Transcriptase (1 μl) according to the manufacturer’s protocol (Fermentas). The cycling protocol for 20 μl reaction mix was 5 min at 65 °C, followed by 60 min at 42 °C, and 5 min at 70 °C to terminate the reaction.

**Second strand cDNA synthesis**

The RT-PCR reaction was carried out in a total volume of 25 μl containing PCR master mix (12.5 μl), FWD and REV specific primers (each 0.5 μl) and cDNA as a template (1 μl) and nuclease free water (10.5 μl). PCR conditions were run as follows: general denaturation at 95 °C for 3 min, 1 cycle, followed by 40 cycles of 95 °C for 20 s; annealing temperature (63 °C for GAPDH and 52 °C for CYP3A2) for 30 s; elongation: 72 °C for 1 min and 72 °C for 5 min. The products of RT-PCR were separated on 1.5% agarose gels containing ethidium bromide and visualized using Gel DOC 2000 system (Bio-Rad). The specific primers for Ratus CYP 3A2 and GAPDH were designed and manufactured by CinnaGen (CinnaGen Co. Tehran, Iran) (Shibata et al., 1999; Wauthier et al., 2006). Primers pairs for RT-PCR were as depicted in Table 1.

**Statistical analysis**

All results are presented as means ± SD. For kinetic studies the Michaelis-Menton constant (Km) and the maximum velocity (Vmax) were obtained. Differences between the amount of the produced metabolite in various study groups and other numerical data were analyzed with one-way ANOVA followed by a Bonferroni test, using Graph Pad Prism 4.00, Graph Pad Software. Data was considered at the significance of 2α = 0.05.

**Results**

**Atorvastatin protected from diabetes-induced increase of BUN, creatinine, daily urine volume and urine protein**

A statistically significant decrease in body weight gain (BWG) and remarkable increase in the serum level of BUN and creatinine were recorded in diabetic rats when compared to control group. Those diabetic animals which were treated with
atorvastatin for 2 weeks, showed a marked therapeutic effect on both BWG and also on renal function testing biomarkers of BUN and creatinine levels (Table 2). Two weeks atorvastatin administration in the non-diabetic rats resulted in a non-significant changes in comparison with the control group. The urine volume and protein level were markedly elevated in the non-treated diabetic rats while atorvastatin-received diabetic rats showed remarkable reduction of both biomarkers (Table 2).

**Atorvastatin showed antioxidant effects on diabetes-induced oxidative and nitrosative stress**

To evaluate the antioxidant status in renal tissues following diabetes induction and also impact of atorvastatin treatment on diabetes-induced oxidative/nitrosative stress, the level of lipid peroxidation end product (MDA), total nitrite/nitrate concentration and total thiol molecules were measured. Results indicated that diabetes markedly elevated the MDA and NO concentration, while enhanced the renal glutathione depletion as the TTM level dropped significantly. Two consecutive weeks’ atorvastatin administrations in the diabetic rats resulted in a remarkable decline in MDA and NO concentrations and reversed the diabetes-induced glutathione depletion. Atorvastatin administration in the non-diabetic animals resulted in no significant changes in antioxidant status (Fig. 1A–C).

**Atorvastatin increased the hydroxylation of testosterone by renal microsomes**

Experimentally-induced diabetes resulted in a significant increase in testosterone hydroxylation without remarkable changes in enzyme affinity toward the substance as shown with non-significant elevation of Km value in the non-treated diabetic animals (Table 3). Atorvastatin-received diabetic animals showed 2 and 1.5-fold increase in the 6-β-hydroxytestosterone production when compared to the control and non-treated diabetic groups, respectively (Fig. 2). Km value for the 6-β-hydroxytestosterone production in the atorvastatin-received animals slightly but statistically significant was declined. Interestingly, the animals in the atorvastatin-treated non-diabetic group also showed a marked elevation in the Vmax value recorded for the 6-β-hydroxytestosterone production without any significant alteration in km value (Table 3).

**Atorvastatin improved the diabetes-induced tubular and capsular dilation in the kidneys**

Pathological examinations revealed that unlike the control group which showed a normal structure of kidney (Fig. 3A), the diabetic animals showed an abnormal kidney architecture that was characterized by a severe dilatation of capillaries in the cortex of kidneys, tubular dilatation, urinary space dilation, interstitial edema, and cystic tubules (Fig. 3B). Atorvastatin administration in diabetic animals resulted in a marked reduction of size and the number of diabetes-induced tubular dilatation (Table 4), and also remarkable reduction in the cystic tubules (Fig. 3C). Additionally, atorvastatin administration remarkably reduced the urinary space dilation and

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**Fig. 1 – Effect of atorvastatin on diabetes-induced MDA (A), and NO level (B) and diabetes-reduced TTM level (C) in the kidney tissue; data is given as mean ± SD (n = 8). * represents a statistically significant difference between the control versus non-treated diabetic rats and # represents a significant difference between the non-treated and treated diabetic groups.**
ment con examinations indicated the atorvastatin’s therapeutic effects markers, renal function testing assays and histopathological and non-diabetic animals. Moreover, oxidative stress bio-
tion up-regulated the aforementioned enzyme in the diabetic
the renal microsomes. Additionally, atorvastatin administra-
by renal microsomes in diabetic and non-diabetic rats.

Fig. 2 – Effect of atorvastatin on Testosterone hydroxylation by renal microsomes in diabetic and non-diabetic rats.

**Table 3 – Effect of atorvastatin on enzyme kinetics of testosterone hydroxylation by the renal microsomes in diabetic rats (n = 8).**

<table>
<thead>
<tr>
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<th>C</th>
<th>D</th>
<th>AD</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vₘₐₓ (pmol/min/mg)</strong></td>
<td>21.6 ± 1.5</td>
<td>34.7 ± 1.3³</td>
<td>45.0 ± 2.3⁴</td>
<td>42.4 ± 1.2²</td>
</tr>
<tr>
<td><strong>Kₘ (μmol/l)</strong></td>
<td>8.7 ± 2.8</td>
<td>11.3 ± 1.8¹</td>
<td>7.1 ± 1.9⁴</td>
<td>8.8 ± 1.1</td>
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<tr>
<td><strong>R²</strong></td>
<td>0.76</td>
<td>0.93</td>
<td>0.82</td>
<td>0.94</td>
</tr>
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</table>


* Significant differences (P < 0.05) between the control and non-treated diabetic groups.

# Significant differences between non-treated and atorvastatin-treated diabetic groups.

Discussion

The present study showed that the experimentally-induced diabetes (type–I) in rats resulted in a marked up-regulation of CYP 3A2 in renal tissue. At the same time, atorvastatin-receiving diabetic and non-diabetic rats showed a remarkable up-regulation in the expression of CYP 3A2 in the renal tissue. We found the highest up-regulation of CYP 3A2 in the kidneys of diabetic rats

Atorvastatin up-regulated the expression of Cytochrome P450 3A2 in the kidneys of diabetic rats

Streptozotocine-induced diabetes resulted in a marked up-regulation of CYP 3A2 in renal tissue. At the same time, atorvastatin-received diabetic and non-diabetic rats showed a remarkable up-regulation in the expression of CYP 3A2 in the renal tissue. We found the highest up-regulation of CYP 3A2 in the atorvastatin-treated diabetic rats (Fig. 4).

Our daily based observations and blood glucose measure-
ment confirmed that the experimentally-induced diabetes type I with STZ worked correctly as 48 h after STZ administra-
tion and during the study period, we observed a high blood glucose level accompanied with polydipsia and polyuria. Those non-treated diabetic rats showed a significant weight
losing which may be explained by extremely water losing and dehydration. Due to the diabetes-induced hyperglycemia, osmotic diuresis is created which in turn results in dehydration and weight loss. At the same time, because of insulin deficieny/resistance in diabetic patients protein degradation is increased and utilizing the stored fat is accelerated that these two results in rapid body weight loss (Goodman, 2003). It has been shown that insulin deficieny/resistance leads to suppression of PI3K/Akt signaling and consequently to activation of caspase-3 and the ubiquitin-proteasome proteolytic pathway causing muscle protein degradation (Wang et al., 2006). It has been previously reported that in addition of impaired insulin signaling, increased glucorticoid levels also is needed for muscle degradation and weight losing (Hu et al., 2009). The muscle wasting or in other word the weight loss may be also related to chronic kidney injuries which is occurred in diabetic patients. Among the others nephropathy is one of the serious complications of diabetes and when it is become chronic, it may help to muscle wasting signals to be more activated.

There are several genetic and environmental factors, which could be considered as crucial factors in pathogenesis of diabetic nephropathy (DN), promoting the formation of advanced glycation end products (AGEs), leading the excessive level of reactive oxygen species (ROS) generation and also kidney inflammation (Stanton, 2011; Tesch and Lim, 2011). Our renal function testing assays including determination of serum level of creatinine and blood urea nitrogen (BUN) revealed that both biomarkers elevated dramatically in non-treated diabetic animals. These two biomarkers along with polyuria and hyperglycemia confirmed diabetes induction and also diabetic nephropathy. The histopathological examinations also showed diabetic nephropathy as in the STZ-induced non-treated diabetic rats, thickening of the glomerular basement membrane, urinary space dilation, interstitial edema, and cystic tubules represented an abnormal and injured renal architecture.

Previously we showed that in diabetic rats, one of the most active systems in drug and xenobiotics metabolizing is activated in the liver (Malekinejad et al., 2012). We also showed that the up regulation of the drug metabolizing enzymes is not limited to mRNA level but also its enzymatic function in the biotransformation of natural substrate augmented (Malekinejad et al., 2014). Although the current understanding about the drug metabolism is based on studies

interstitial edema. Those non-diabetic animals, which received atorvastatin, showed mild hydropic degeneration and protein casts in cortical tubules (Fig. 3D).
which are indicating that the hepatic biotransformation is the main metabolism approach, there are however increasing evidences showing that the kidney also metabolizes many drugs, hormones, and xenobiotics (Lohr et al., 1998). It has been reported that in some cases such as glycination of benzoic acid, biotransformation occurs at a faster rate in the kidney than in the liver (Poon and Pang, 1995). In the light of previous studies in terms of alterations in biotransformation capacities in diabetic cases versus control animals, it was the motive of the current study to investigate any enzymatic activity, the expression profile of Cytochrome P450 3A2 in the kidney of control and diabetic rats. We showed that the experimentally-induced diabetes up-regulated the expression of CYP 3A2 in the kidneys like as liver. The up regulation of CYP 3A2 at mRNA level later was supported by increasing the testosterone conversion assay, suggesting that diabetes not only up

<table>
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<tr>
<th>Groups</th>
<th>Urinary space dilation (µM)</th>
<th>Number of dilated tubules$^a$</th>
<th>Tubule dilation (µM)</th>
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<tr>
<td></td>
<td>Cortex</td>
<td>Medulla</td>
<td>Cortex</td>
</tr>
<tr>
<td>C</td>
<td>6.4 ± 1.5</td>
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<td>7.9 ± 2.6</td>
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<tr>
<td>D</td>
<td>10.7 ± 1.3$^*$</td>
<td>27.2 ± 3.4$^*$</td>
<td>26.7 ± 1.6$^*$</td>
</tr>
<tr>
<td>AD</td>
<td>6.1 ± 1.1$^*$</td>
<td>10.8 ± 1.2$^*$</td>
<td>15.0 ± 4.8$^*$</td>
</tr>
<tr>
<td>A</td>
<td>5.8 ± 0.9</td>
<td>2.0 ± 0.8</td>
<td>8.5 ± 2.4</td>
</tr>
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</table>

$^a$ The number of dilated tubules were counted collectively in 30 microscopic fields from at least 3 slides for each single animal of study groups.

$^*$ Significant differences ($P < 0.05$) between the control and non-treated diabetic groups.

$^*$ Significant differences between non-treated and atorvastatin-treated diabetic groups.

Table 4 – Effect of atorvastatin on diabetes-induced histopathological damages in the kidneys.

Fig. 3 – Photomicrograph of rat's kidney; (A) control; normal feature of kidney, (B) diabetic group; the kidney tissue with protein Casts (arrow), hydropic degeneration (arrow head), interstitial edema (bold arrow), cystic tubules (hollow arrow) and dilation of urinary space (star) are demonstrated, (C) the number of dilated bowman capsules and tubules remarkably was reduced in the diabetic atorvastatin-received rats and (D) atorvastatin non-diabetic animals showed only a few cortical tubules with protein casts and mild hydropic degeneration. Hematoxylin & Eosin staining, 400× and scale = 200 µm.
regulated the CYP 3A2 transcriptionally but its enzymatic activity also augmented.

The second part of this study devoted to uncover the effect of atorvastatin administration on diabetes-induced alteration in renal functions, structure and also in the expression of CYP 3A2 and its enzymatic activity in testosterone converting assay. Our results showed that atorvastatin administration in the diabetic rats reduced the diabetes-elevated serum level of renal function biomarkers of BUN and creatinine, significantly, suggesting a protective effect of atorvastatin in diabetic patients. Previous studies also showed that one of the key factors in the pathogenesis of diabetes in particular in STZ-induced experimental diabetes is elevation of the ROS generation under glycation reaction that occurs in various tissues including the liver, pancreatic beta cells and kidney (Srinivasan and Menon, 2003; Giacco and Brownlee, 2010). At the same time, although the role of NO at the physiologic level is participating in hemostasis, vascular tone regulation in the kidneys, the excessive generation of NO which occurs during the diabetes, however contributes in the development and progression of diabetes-induced nephropathy (Dellamea et al., 2014). The first part of our study also confirmed the fact that oxidative and nitrosative stress are induced in STZ-induced diabetes and biomarkers including elevation in lipid peroxidation rate and NO content and reduction in glutathione level in renal tissue were noticed. Further findings of the current study uncovered the therapeutic effect of atorvastatin on diabetes-induced oxidative and nitrosative stress. An anticancer effect of atorvastatin in nitrosamine-induced rat bladder cancer, which was mediated via its antioxidant and anti-inflammatory properties have been recently reported (Parada et al., 2012). Mechanism of therapeutic effect of statins in diabetic patients in addition of their lipid lowering property, related to the restriction of oxygen free radical production by high level of glucose (Christ et al., 2002). Statins inhibit the diabetes-induced oxidative stress in the vascular system via inhibition of Rac-1 through a reduction in geranyl pyrophosphate (Vecchione et al., 2007).

The histopathological examinations supported the biochemical changes as we found diabetic nephropathy characters including urinary space dilation, and tubular dilation that all indicating diabetes-induced renal damage. The atorvastatin administration markedly reduced the diabetes-induced damages in the kidney, although it was not able to fully recover those injuries. Our finding is supported with recently published report indicating that that 10 mg/kg atorvastatin for 24 weeks in human diabetic patients reduced the podocyte injuries, significantly (Takemoto et al., 2013). The renoprotective effect of atorvastatin may be related to its chemical structure which permits to locate in cell membrane and influence the intracellular signaling such as podocyte cytoskeleton rearrangement (Shibata et al., 2006).

We previously showed that the STZ-induced diabetes resulted in up-regulation of CYP 3A2 in the liver of rats

Fig. 4 – Effect of atorvastatin on: (a) CYP 3A2 and (b) GAPDH mRNA levels in the kidney of diabetic and non-diabetic rats; the levels of CYP 3A2 mRNA were evaluated by semi-quantitative RT-PCR, and (c) represents the density of CYP 3A2 mRNA in the kidney that were measured by densitometry and normalized to GAPDH mRNA expression level. Results were expressed as integrated density values (IDV) of CYP 3A2 mRNA level.
(Malekinejad et al., 2012). Another in vitro study also demonstrated that high glucose induces the ROS production and apoptosis in cultured mouse podocytes through the up-regulation of CYP 4A (Eid et al., 2009). Our results in the current study also confirmed and extended the up-regulation of CYP 3A2 to the renal tissue of the diabetic rats as both the expression of CYP 3A2 at mRNA level and also its enzymatic activity through testosterone conversion assay were enhanced significantly. Although the up-regulation of CYP 3A2 in the renal tissue of diabetic rats is reported for the first time, however, induction of CYPs in diabetic mouse and hamster have already been demonstrated (Chen et al., 1996; Dong et al., 2009). Atorvastatin administration in the diabetic and non-diabetic rats not only did not reduce the diabetes-induced CYP 3A2 expression but also resulted in significantly more expression of aforementioned gene in renal tissue of diabetic and non diabetic animals. To interpret this finding one should note that there are so variation between the different statins and also between various cells and tissues in responding to statins in terms of gene expression. For example, atorvastatin dose-dependently up-regulated the expression of CYP 2C9 in HepG2 cells, while at the same dose levels down-regulated the same gene in the human vascular endothelial cell line (Bertrand-Thiebault et al., 2007). The same report indicated that lovastatin acts on the expression of CYP 2C9 exactly in reverse order that atorvastatin does. Another study which took place on human patients showed that atorvastatin administration increased remarkably CYP 3A4 and 3A5 mRNA expression in peripheral blood mononuclear cells of hypercholesterolemic patients (Willrich et al., 2013). One more explanation for different responses of various cells and tissues to atorvastatin may be related to its biotransformation profile. It has been documented that atorvastatin and its active metabolites are ligands of pregnane X receptor (PXR), but not of constitutive androstane receptor (CAR), which are responsible for the induction of drug metabolizing enzymes and transporter (Kocarek et al., 2002). Interestingly, only the parahydroxy metabolite showed significantly impaired induction of PXR-regulated genes including CYPs, indicating that the variation between cells and organs in response to atorvastatin administration may be influenced by the formed active metabolite (Hoffart et al., 2012).

In conclusion, evidence is provided for the up-regulation of CYP 3A2 at the mRNA and enzymatic levels by atorvastatin in diabetic and non-diabetic rats. This study also demonstrated that atorvastatin exert a nephroprotective effect in STZ-induced diabetic rats, which characterized by improving the diabetes-induced nephropathy and renal function biomarkers including BUN and creatinine levels. The antioxidant effect of atorvastatin also was shown in the renal tissue of diabetic rats.

Conflict of interest

None declared.

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References


