

Original Article

Protective effects of rosuvastatin against hyperglycemia-induced oxidative damage in the pancreas of streptozotocin-induced diabetic rats

Samira Yazdanimehr¹, Mohammad Taghi Mohammadi^{2,3*} 

1. Students' Research Committee, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Exercise Physiology Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Department of Physiology and Biophysics, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

Abstract

Introduction: According to the powerful antioxidant effects of rosuvastatin, the present study aimed to examine the protective effects of rosuvastatin against oxidative damage of diabetic pancreas by potentiation of the antioxidant capacity in streptozotocin-induced diabetic rats.

Methods: Experiment was performed in four groups of male Wistar rats (n=6 in each group): normal, diabetic and two treatment groups (normal and diabetic rats treated with rosuvastatin). Rats were made diabetic by a single intravenous injection of streptozotocin (40 mg/kg) at the beginning of study. Treatment groups received orally rosuvastatin at dose of 10 mg/kg/day. After eight weeks, the pancreas tissues were removed under deep anesthesia. After tissue homogenization, the contents of glutathione and malondialdehyde (MDA) as well as superoxide dismutase (SOD) activity were assessed by biochemical methods.

Results: Blood glucose of diabetic rats was above 350 mg/dl. The MDA content of the homogenized pancreas significantly increased in diabetic rats by 92%. Diabetes also decreased the content of glutathione (32%) as well as SOD activity (68%) of pancreas tissues. Treatment with rosuvastatin noticeably decreased the MDA levels of diabetic pancreas (90%). Moreover, rosuvastatin significantly increased the glutathione content (21%) and SOD activity (67%) of pancreas tissues in treated diabetic rats.

Conclusion: Our findings reveal that rosuvastatin is able to attenuate the uncontrolled hyperglycemia-induced oxidative damage of pancreas through potentiation of the antioxidant defense system.

Keywords:

Diabetes mellitus;
Rosuvastatin;
Hyperglycemia;
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*Correspondence to:

M.T. Mohammadi

Tel/Fax: +98-2182483419

Mobile: +98-9127713583

Email:

Mohammadimohammadt@bmsu.ac.ir

Introduction

Diabetes mellitus is a chronic metabolic disease with a high prevalence in worldwide. It is estimated that

the prevalence of diabetes will rise to 366 million in 2030 (Rathmann and Giani, 2004). Impaired insulin synthesis and secretion due to damage of pancreatic β -cells (type 1 diabetes) and decreased responsiveness of peripheral insulin receptors due to

insulin resistance (type 2 diabetes) are the main reasons of diabetes (Poitout, 2008; Yoon and Jun, 1999). The glucose toxicity in the β -cell of pancreatic islets is more common in people with type 2 diabetes because most patients with type 2 diabetes have abnormally elevated postprandial glucose concentrations that continually damage the remaining β -cells (Robertson and Harmon, 2006). Hyperglycemia is the main symptom of diabetes and it has been reported a direct correlation between chronic hyperglycemia and generation of reactive oxygen species (ROS), (Maritim et al., 2003). Exposure to high glucose levels has been reported that enhances intra-islet levels of peroxide (Tanaka et al., 2002). According to measurements of the oxidative stress markers in patients with type 2 diabetes, diabetic patients show elevated markers for oxidative tissue damage and oxidants in the tissues, such as peroxides and oxidation of DNA bases (Reus et al., 2016; Yang et al., 2011). There are several pathways, including polyol and hexosamine pathways and also activation of protein kinase C and NADPH-oxidase, through which chronic hyperglycemia can lead to the ROS generation within the islets of pancreas (Robertson and Harmon, 2006). Moreover, weakening of the antioxidant defense system of pancreatic islets is occurred under hyperglycemic conditions (Kakkar et al., 1998). It has been reported that diabetes substantially decreases the activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) as well as the mRNAs and proteins levels of these antioxidant enzymes in pancreatic islets (Kakkar et al., 1998; Robertson and Harmon, 2006; Tanaka et al., 2002).

Statins, 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, such as rosuvastatin have the pleiotropic effects independent of lipid-lowering actions in several pathological states (Arboix et al., 2010; Castro et al., 2008). Previously, improved endothelial function and reduced oxidative stress by rosuvastatin have been reported in streptozotocin-induced diabetic mice through a cholesterol-lowering independent mechanism (Giunti et al., 2010). Deng et al. (2015) showed that rosuvastatin decreases contrast-induced nephropathy through modulation of nitric oxide, oxidative stress, apoptosis and inflammatory responses in diabetic rats. Rosuvastatin also induces vasoprotection through increasing of nitric oxide bioavailability, which

is possibly achieved by its inhibition on ROS production (Tian et al., 2011). In another study Calkin et al. (2008) indicated the anti-atherosclerotic effects of rosuvastatin in a model of atherosclerosis accelerated by diabetes that are independent of its lipid lowering effects. They also reported that treatment with rosuvastatin was associated with reduced accumulation of advanced glycation end-products (AGE) and AGE receptor (RAGE) in plaques. Besides the beneficial effects of statins, dual effects of rosuvastatin on glucose homeostasis have been reported during diabetes. It has been demonstrated that rosuvastatin improves insulin sensitivity but impairs β -cells function (Salunkhe et al., 2016). Also, association between increased risk of diabetes with statins and impaired insulin sensitivity and insulin secretion has been reported, previously (Laakso and Kuusisto, 2017).

According to the previous reporters, weakening of the antioxidant defense system of pancreatic islets and consequent oxidative damage to the pancreatic β -cells has been occurred during diabetes (Coskun et al., 2005). Hence, because of the beneficial pleiotropic effects of rosuvastatin, the present study aimed to evaluate whether rosuvastatin attenuates the oxidative stress of pancreas and potentiates the antioxidant defense system of pancreas in streptozotocin-induced diabetic rats.

Materials and methods

Animals

All procedures of the study were approved by the Institutional Care and Use of Animals Committee of the University of Baqiyatallah Medical Sciences, and were performed in accordance with accepted standards of animals use and care. The ethical code for the present research was IR.BMSU.REC.1396.170. We used male Wistar rats, weighing 280-320 g, obtained from the animal house facility center of Baqiyatallah University of Medical Sciences. Before procedures, the rats were allowed to acclimatize to the new situation with controlled temperature (22-24 °C), humidity (40-60%), light period (07.00-19.00) and also free access to the rat chow and water.

Induction of diabetes

An intravenous injection of streptozotocin (Sigma

Aldrich, USA) was used for induction of type 1 of diabetes. In brief, under light anesthesia using ethyl ether, diabetes was induced by an intravenous injection of 40 mg streptozotocin per kg body weight of rats in lateral tail vein. Five days after injection of streptozotocin, blood glucose levels were tested to confirm diabetes state and the rats with blood glucose levels over 350 mg/dl were considered as diabetic animals (Mohammadi et al., 2014; Pirmoradi et al., 2014). Blood glucose levels at the beginning and termination of experiment were measured using a commercial kit (Pars Azmoon Company, Tehran, Iran) by an enzymatic colorimetric method according to the manufacture protocols.

Rosuvastatin treatment

Rosuvastatin (AstraZeneca, UK) was orally administered at a dose of 10 mg/kg/day by oral gavage for eight consecutive weeks. The dose of rosuvastatin was decided according to the study of Deng et al. (2015). Rosuvastatin was dissolved in distilled water.

Experimental protocols

To perform the study, the rats were randomly divided into four groups of equal numbers (six rats in each group) as follows: normal group (normal healthy rats that used as normal control), rosuvastatin-treated normal rats (normal healthy rats that received orally rosuvastatin at a dose of 10 mg/kg/day for eight weeks), diabetic control (diabetic rats that used as diabetic control) and rosuvastatin-treated diabetic rats (diabetic rats that received orally rosuvastatin at a dose of 10 mg/kg/day for eight weeks). The rats of normal and diabetic control groups were administered orally distilled water as vehicle at same volume which the rosuvastatin-treated normal and diabetic rats received rosuvastatin solution.

Tissue preparation

Under deep anesthesia using ethyl ether, the pancreas tissues were rapidly removed, washed in an ice-cold phosphate buffer saline (PBS), immersed in liquid nitrogen and finally kept on -80 °C until biochemical analysis. Then, the tissues were weighed and homogenized 1:10 in ice-cold PBS. The homogenates were centrifuged at 14000 g for 15 minutes at 4 °C. After centrifugation, the supernatants were removed and used for measurement of

malondialdehyde (MDA) and glutathione as well as superoxide dismutase (SOD) activity.

Determination of glutathione content

In the present study, the method of Tietz (1969) was used to measure the content of glutathione. "Cellular protein was precipitated by addition of 5% sulfosalicylic acid and removed by centrifugation at 2000 g for 10 minutes. Glutathione in the supernatant was assayed as follows: 100 µl of the protein-free supernatant of the cell lysate, 800 µl of 0.3 mM Na₂HPO₄ and 100 µl of 0.04% 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in 0.1% sodium citrate. The absorbance of DTNB was monitored at 412 nm for 5 minutes. A standard curve of glutathione performed and sensitivity of measurement was determined to be between 1 and 100 nMol" (Vani et al., 2016). The glutathione levels were represented as nMol/ml.

Assessment of SOD activity

The activity of SOD was determined using the method designated by Winterbourn et al. (1975) based on the ability of this enzyme to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide. "For the assay, 0.067 M potassium phosphate buffer, pH 7.8 was added to 0.1 M EDTA containing 0.3 mM sodium cyanide, 1.5 mM NBT and 0.1 ml of sample. Then, 0.12 mM riboflavin was added to each sample to initiate the reaction and was incubated for 12 min. The absorbance of samples was read on a Genesys 10 UV spectrophotometer at 560 nm for 5 minutes. The amount of enzyme required to produce 50% inhibition was taken as 1 U and results were expressed as U/ml protein" (Vani et al., 2016).

Determination of MDA

The method of Satoh (1978) was used to measure the content of MDA, as an index of oxidative damage. "The 0.5 ml of tissue homogenate was added to 1.5 ml of 10% trichloroacetic acid, vortexed and incubated for 10 min at room temperature. The 1.5 ml of supernatant and 2 ml of thiobarbituric acid (0.67%) were added and placed in a boiling water bath in sealed tubes for 30 min. The samples were allowed to cool to room temperature. The 1.25 ml of n-butanol was added, vortexed and centrifuged at 2000 g for 5 min. The resulting supernatant was removed and

measured at 532 nm on a spectrophotometer. MDA concentrations were determined using 1,1,3,3-tetraethoxypropane as standard. The content of MDA was ultimately represented as nMol/ml¹⁷ (Arjmand Abbassi et al., 2016).

Statistical analysis

The statistical analysis was performed using SPSS, version 22. All data in the present study were expressed as mean±SEM. Data were analyzed with one-way ANOVA followed by Tukey post-hoc test. All

states, *P* value of <0.05 was considered statistically significant.

Results

Effects of rosuvastatin on blood glucose

Blood glucose (mg/dl) of the rats is shown in Figure 1 at the end of the experiment. Treatment with rosuvastatin did not significantly change the value of blood glucose in normal treatment group (131±8 mg/dl) compared with normal control group (144±10

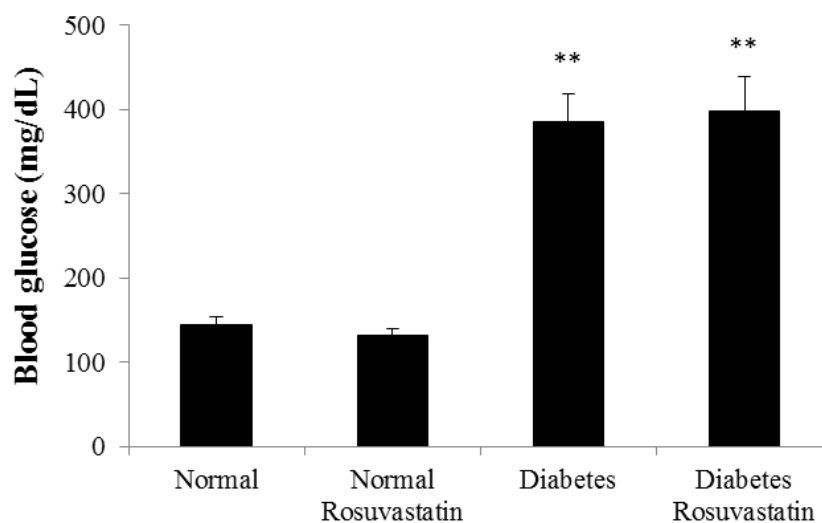


Fig.1. Representative changes of blood glucose (mg/dl) in normal and diabetic groups at termination of the study. The rats received daily rosuvastatin (10 mg/kg, orally) for eight weeks. (n=6 for each group). All values are presented as mean±SEM. ** as significant difference compared to normal group (*P*<0.01).

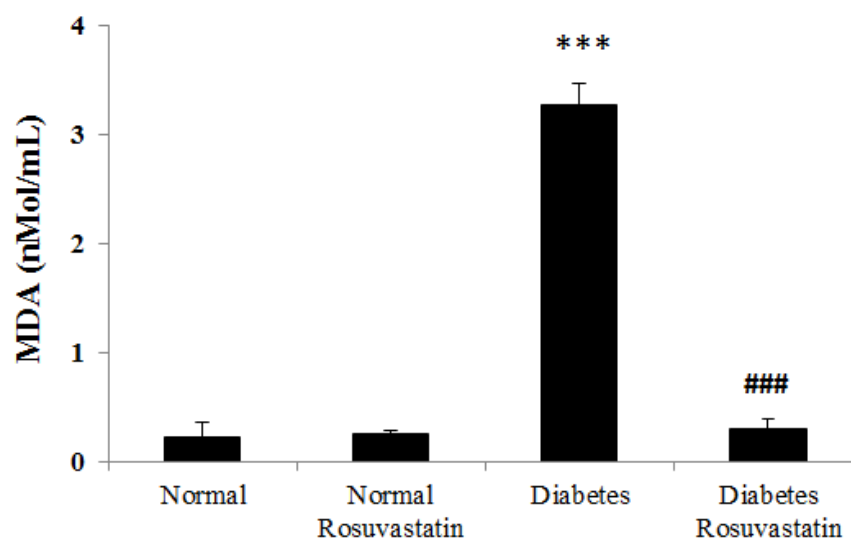


Fig.2. Malondialdehyde (MDA) concentration (nMol/ml) of the pancreas tissues in normal and diabetic groups at termination of the experiment. The rats received daily rosuvastatin (10 mg/kg, orally) for eight weeks. (n=6 for each group). All values are presented as mean±SEM. *** as significant difference compared to normal group (*P*<0.001). ### as significant difference compared to diabetic group (*P*<0.001).

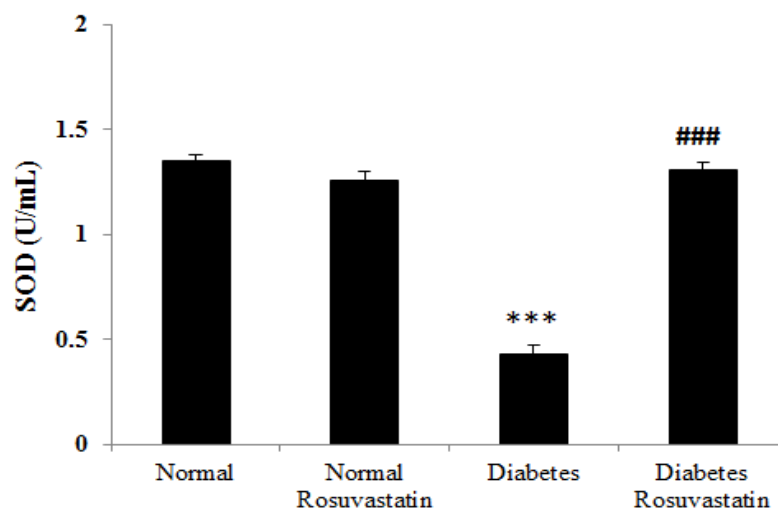


Fig.3. Superoxide dismutase (SOD) activity (U/ml) of the pancreas tissues in normal and diabetic groups at termination of the experiment. The rats received daily rosuvastatin (10 mg/kg, orally) for eight weeks. (n=6 for each group). All values are presented as mean±SEM. *** as significant difference compared to normal group ($P<0.001$). ### as significant difference compared to diabetic group ($P<0.001$).

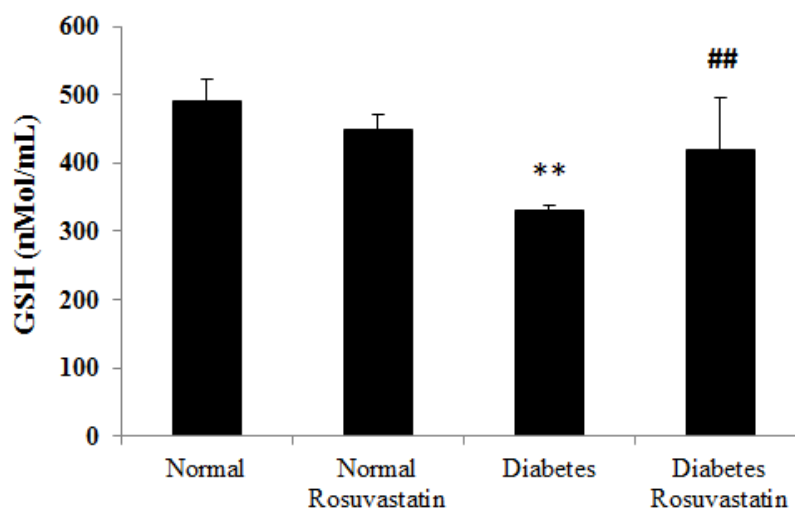


Fig.4. Glutathione (GSH) content (nMol/ml) of the pancreas tissues in normal and diabetic groups at termination of the study. The rats received daily rosuvastatin (10 mg/kg, orally) for eight weeks. (n=6 for each group). All values are presented as mean±SEM. ** as significant difference compared to normal group ($P<0.01$). ## as significant difference compared to diabetic group ($P<0.01$).

mg/dl). Streptozotocin injection in diabetic groups increased the values of blood glucose above 350 mg/dl. Blood glucose of diabetic control group was significantly increased compared to the normal rats (385 ± 33 mg/d, $P<0.01$). Treatment with rosuvastatin did not significantly change blood glucose of the diabetic treatment group (398 ± 41 mg/dl) compared with diabetic control group.

Effects of rosuvastatin on MDA levels

As shown in Figure 2, MDA levels (nMol/ml) of pancreas homogenate in normal group was 0.22 ± 0.13 nMol/ml at the end of the study.

Rosuvastatin treatment did not significantly change the mean value of MDA in normal treatment group (0.26 ± 0.03 nMol/ml) compared with normal control group. The results of diabetic group indicated that diabetes significantly increased the MDA levels in the pancreas of control diabetic group (3.27 ± 0.20 nMol/ml) compared with normal group ($P<0.001$). Treatment with rosuvastatin significantly decreased the levels of MDA in the pancreas homogenate of diabetic treatment group (0.29 ± 0.10 nMol/ml) compared to diabetic control group ($P<0.001$).

Effects of rosuvastatin on SOD activity

The relative activity of SOD in pancreas tissues is presented in Figure 3 at the termination of the experiment. There was no significant difference in SOD activity between normal (1.35 ± 0.03 U/ml) and normal treatment group (1.26 ± 0.04 U/ml). The results of diabetic group indicated that diabetes significantly decreased the activity of SOD in diabetic control group (0.43 ± 0.04 U/ml) compared to normal group ($P < 0.001$). Rosuvastatin-treated diabetic rats showed a significant increase in the mean value of SOD activity (1.31 ± 0.03 U/ml) compared to diabetic control group, ($P < 0.001$).

Effects of rosuvastatin on glutathione concentration

The glutathione level (nMol/ml) of pancreas homogenate is shown in Figure 4 at termination of the experiment. There was no significant difference in glutathione concentration between normal (491 ± 32 nMol/ml) and normal treatment group (448 ± 23 nMol/ml). Diabetes significantly decreased glutathione levels of the pancreas tissues in diabetic control group (330 ± 8 nMol/ml) compared with normal group ($P < 0.01$). Treatment with rosuvastatin significantly enhanced the levels of glutathione in diabetic treatment group (420 ± 76 nMol/ml) compared to diabetic control group ($P < 0.01$).

Discussion

In the present study, diabetic animals showed a considerable increase in the index of oxidative damages (increased MDA levels) in the pancreas tissues after eight weeks. The antioxidant defense system of pancreas tissue also significantly reduced in the diabetic rats that have uncontrolled hyperglycemia. On the other hand, rosuvastatin-treated diabetic rats revealed a significant decrement of oxidative damage in the pancreas tissues (decreased MDA levels) after eight weeks treatment. Treatment with rosuvastatin in diabetic rats for eight weeks also potentiated the antioxidant capacity of pancreas through an increase in the activity of SOD and glutathione levels compared to the untreated diabetic animals.

The findings of the present study indicated that ROS generation and oxidative damage (increased levels of MDA) were noticeably

increased in the pancreas tissues of diabetic rats. The resultant uncontrolled and chronic hyperglycemia leads to ROS generation and oxidative damage for pancreas islets because glucose in high concentrations forms ROS (Robertson and Harmon, 2006). It has been reported that ROS generation and oxidative damage are as the fundamental mechanisms for glucose toxicity in pancreatic islet β -cells during diabetic conditions (Robertson, 2004). Pancreatic β -cells are principally sensitive to oxidative damage due to the low levels in free radicals quenching enzymes (antioxidant defense system) such as SOD, GPx and catalase (Valko et al., 2007). Thus, the islets cells of pancreas are at higher risk of oxidative stress than tissues with greater levels of antioxidant protection. So, the capability of oxidative damage to destroy the β -cells and decline insulin synthesis and secretion is not surprising. Additionally, it has been repeatedly reported a decrease in the antioxidant capability of islets β -cells as a result of increased free radical generation in experimental diabetes (Coskun et al., 2005; Valko et al., 2007). Our results revealed that diabetic rats showed significantly decreased the capacity of the pancreatic antioxidant defense system (decreased SOD activity and glutathione levels) against ROS generation during uncontrolled hyperglycemia. This means that the weakened cellular antioxidant status during hyperglycemia increases the susceptibility of pancreatic islets β -cells to the oxidative damage in diabetes with high oxidative stress. According to our results, Coskun et al. (2005) showed that tissue MDA significantly increases in the diabetic states in accompany with a decrease in the antioxidant enzyme activities of SOD and GPx. Additionally, Yoshida et al. (1995) reported that the intracellular content of glutathione decreases in diabetic condition because the activity of γ -glutamyl cysteine ligase, the rate-limiting enzyme for glutathione synthesis, decreases under diabetic states.

According to our results, treatment with rosuvastatin considerably decreased the ROS production and oxidative damage (decreased levels of MDA) of the pancreas tissues in

treated diabetic rats. Previously, the antioxidant properties of rosuvastatin have been demonstrated in several tissues during different pathological states (Deng et al., 2015; Heeba and Hamza, 2015). Recently, Heeba and Hamza (2015) reported that rosuvastatin administration attenuates diabetes-induced reproductive damage in male rats through suppression of ROS generation and oxidative stress. Based on the findings of Deng et al. (2015) treatment with rosuvastatin ameliorates contrast-induced nephropathy via modulation of ROS generation and oxidative stress in diabetic male rats. Several pathways have been demonstrated that statins by them suppress ROS production and oxidative stress (Calkin et al., 2008; Deng et al., 2015). It has been reported that interruption of the NADPH-oxidase pathway by rosuvastatin results in reduced ROS production (Heeba and Hamza, 2015). Also, recent studies have demonstrated statin through effect on AGE/RAGE pathway in diabetes may reduce AGE accumulation and thereby reduce activation of NADPH-oxidase and ROS accumulation (Calkin et al., 2008). Our findings also revealed that rosuvastatin treatment increased the glutathione content and SOD activity of pancreas tissues in treated diabetic rats. Although the mechanisms that rosuvastatin can increase the SOD activity are unknown, it is suggested that rosuvastatin may has increased the total content of enzyme by increasing the gene expression levels of SOD. Also, about the glutathione content it is possible that the activity of γ -glutamyl cysteine ligase, the rate-limiting enzyme for glutathione synthesis, has been enhanced by rosuvastatin. Glutathione is a cofactor of several detoxifying enzymes against oxidative stress such as GPx and also glutathione scavenges hydroxyl radical and singlet oxygen directly (Valko et al., 2007). SOD has been shown to protect the cells against oxidative stress by eliminating superoxide anion (Rowley and Patel, 2013). Moreover, SOD is an important antioxidant enzyme that quenches the superoxide anions in mitochondrial matrix (Rowley and Patel, 2013). Therefore according to our results, a decrease in the levels of ROS and oxidative damage in the pancreas tissues of

rosuvastatin-treated diabetic rats may, in part, be by potentiation of the antioxidant defense system of diabetic pancreas. Moreover, the anti-inflammatory functions of rosuvastatin have been reported during diabetes (Heeba and Hamza, 2015; Kata et al., 2016). Thus according to the previous reports, it is proposed that inhibition of inflammation in the islets of pancreas by rosuvastatin be another mechanism of the β -cells protection in diabetes.

Ultimately, treatment with rosuvastatin did not significantly change the blood glucose of treated normal and diabetic rats because rosuvastatin is a HMG-CoA reductase inhibitor. These results are in accordance with previous findings (Mooradian et al., 2005), and this means that rosuvastatin attenuates the oxidative damage of diabetic pancreas independent of its lipid lowering effects or any effect on the blood glucose of diabetic rats. Additionally, it has been reported that rosuvastatin has dual effects on the glucose homeostasis during diabetes (Laakso and Kuusisto, 2017). It has been demonstrated that rosuvastatin improves the sensitivity of peripheral insulin receptor but impairs β -cells function and insulin secretion (Salunkhe et al., 2016). Hence, due to two dual opposite effects, it is suggested that rosuvastatin in the present study did not change the blood glucose of diabetic rats. Because of some limitations (such as lack of the method to measure the levels of insulin release in our department) in the present study, we can't assess the mechanisms that rosuvastatin stimulates the levels of insulin synthesis or secretion by rosuvastatin. Therefore, other studies need to clarify the different pathways that rosuvastatin affects the insulin synthesis or secretion by pancreatic β -cells.

Conclusion

The results of the present study indicate that treatment with rosuvastatin, an inhibitor of HMG-CoA reductase, attenuates the uncontrolled hyperglycemia-induced oxidative damage of pancreas tissue in diabetic state. Rosuvastatin decreases this damage by potentiation of the antioxidant capacity of diabetic pancreas. Our results

suggest the effective therapeutics of rosuvastatin for pancreatic islets β -cells against the oxidative damage in diabetes. Finally, our findings propose a new attitude to prevent the progression of diabetes by elimination of the harmful effects of hyperglycemia-induced tissue oxidative damage in pancreatic β -cells.

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Conflict of interest

The authors declare that they have no competing interests.

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