RESEARCH ARTICLE

Effect of atorvastatin on interleukins and prostaglandin E2 in the kidney of type 1 diabetic rats

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ABSTRACT. ***Objective:*** **The aim of the study was to evaluate a possible effect of atorvastatin on renal interleukins (ILs) and prostaglandin E2 (PGE2) in type 1 diabetic rats.** ***Methods:*** **Thirty-two male rats from a local Wister-derived strain were included in this prospective study and were classified into four groups. Each group consisted of eight animals: Group 1, non-diabetic negative controls; Group 2, diabetic positive controls; Group 3, non-diabetic rats receiving atorvastatin for 4 weeks; and Group 4, diabetic rats receiving atorvastatin for 4 weeks. At the end of the designated period, the animals were sacrificed by cervical dislocation, and the kidneys were excised and homogenized to determine the level of IL-1β, IL-6, IL-10, and PGE2. The study duration was from June 2015 to May 2016 at Al-Ahlyya Amman University, Amman, Jordan.** ***Results:*** **In the kidneys of rats with streptozotocin-induced diabets, the levels of cytokines IL-1β, IL-6, IL-10, and PGE2 were significantly elevated above those of the control group. This clearly showed a detrimental effect of diabetes on the kidney. Treatment of diabetic rats with atorvastatin caused a decrease in all evaluated cytokines to levels near control values.** ***Conclusion:*** **Our data suggest that atorvastatin has the potential to protect or attenuate diabetes-induced renal injury. However, the possible protective effect of atorvastatin should be supported by clinical evidence.**

Key words: cytokines, prostaglandin, atorvastatin, interleukin

In diabetes mellitus, long-term damage, dysfunction, and failure of different body organs are related to uncontrolled diabetic condition, with diabetic nephropathy as one of the major causes of end-stage renal failure and the most frequent cause of mortality in patients with diabetes [1]. Despite the evidence now available about the possible role of oxidative stress in the development of diabetic complications [2], classical antioxidants have failed to show convincing clinical benefits in this area [3]. The interrelationship between the biological actions of cytokines and oxidative stress has been emphasized. Interleukin-1 (IL-1)-induced responses have been reported to occur via modulating redox equilibrium, whereas the biomarkers and the signaling of oxidative stress may arise from, or may be mediated by, cytokine-dependent processes [4, 5].

Inflammatory cytokines involved in the pathogenesis of diabetes play a significant role in the development and progression of several renal disorders, including diabetic nephropathy. The renal effects of inflammatory cytokines are related to the expression of different molecules; intraglomerular hemodynamic abnormalities; alteration of extracellular matrix; and glomerular basement membranes, apoptosis and necrosis, endothelial permeability, oxidative stress, and so forth, determining the development of microvascular diabetic complications, including neuropathy, retinopathy, and nephropathy [6].

IL-1 increases the expression of chemotactic factors and adhesion molecules, enhances vascular endothelial permeability, and stimulates the proliferation of mesangial cells and matrix synthesis [7, 8]. IL-1 was first implicated in the development of diabetic nephropathy when glomerular basement proteins isolated from streptozotocin-induced diabetic male rats had significantly greater macrophage, tumor necrosis factor alpha (TNF-α), and IL-1 production compared with control rats [9]. IL-1 levels are also elevated in kidneys from male Sprague-Dawley rats treated with streptozotocin compared with control rats [10], and renal expression of IL-1 is significantly correlated with urinary albumin excretion [11].

There are conflicting reports about the impact of treatment with statins on the diabetic state [12–16]. New-onset diabetes has been observed in clinical trials and meta-analyses involving statin therapy [17]. On the contrary, there have been claims that rosuvastatin improved the renal function in diabetic subjects through its antioxidant properties [18]. However, in the prolonged condition of diabetes mellitus, the effect of statins on glycemic control is small and unlikely to be clinically important [19].

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Because statins are used as hypolipidemic agents in diabetic subjects, and in view of the conflicting reports on the effect of this group in diabetes, it would be important to evaluate their effect on the changes in the renal biochemical mediators in this disease. The possible effects of atorvastatin were evaluated in the present study.

MATERIALS AND METHODS

The present study was conducted in the Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, Amman, Jordan.

Thirty-two male rats from a local Wister-derived strain weighing 150-180 g were purchased from animal house unit, Jordan University of Sciences and Technology, Irbid, Jordan. All of the animals were kept under observation for 1 week before the study with free access to food and water. Rats were housed at 22 ± 2 °C with a 12 h light-dark cycle. All of the procedures were performed in accordance with the international regulations for the care and use of laboratory animals.

Diabetes was chemically induced by two successive 55 mg/kg intraperitoneal daily doses of streptozotocin (STZ) (Sigma Aldrich Co., St. Louis, MO, USA) in citrate buffer (pH 4.5). The manifestation of diabetes was confirmed within 48 h of injection, which was validated by a blood glucose level above 200 mg% [20].

The rats were divided into the following groups of eight animals each:

– Group 1: non-diabetic rats serving as negative controls
– Group 2: diabetic rats serving as positive controls
– Group 3: non-diabetic rats receiving intraperitoneal atorvastatin (10 mg/kg/day) for 4 weeks
– Group 4: diabetic rats receiving intraperitoneal atorvastatin (10 mg/kg/day) for 4 weeks

At the end of the designated period the animals were sacrificed by cervical dislocation, and the kidneys were excised, washed with ice-cold saline, and stored at −80 °C until homogenized. IL-1β (Cat. #: RLB00), IL-6 (Cat. #: R6000B), IL-10 (Cat. #: R1000) [21], and prostaglandin E2 (Cat. #: KGE004B) [22] were determined in the homogenate by enzyme-linked-immunosorbent serologic assay according to the manufacturer's instructions provided (R&D Systems, Inc., Minneapolis, MN, USA). Total protein in the homogenate was assayed by Lowry's method [23].

All data were expressed as mean ± standard deviation. Descriptive statistical analyses were performed using Microsoft Excel 2010. All analyses and graphics were performed using Graphpad prism, 2007 (Graphpad Software, San Diego, CA, USA). Differences between means were assessed by one-way analysis of variance (ANOVA) followed by Tukey's procedure and were considered statistically significant at $p < 0.05$.

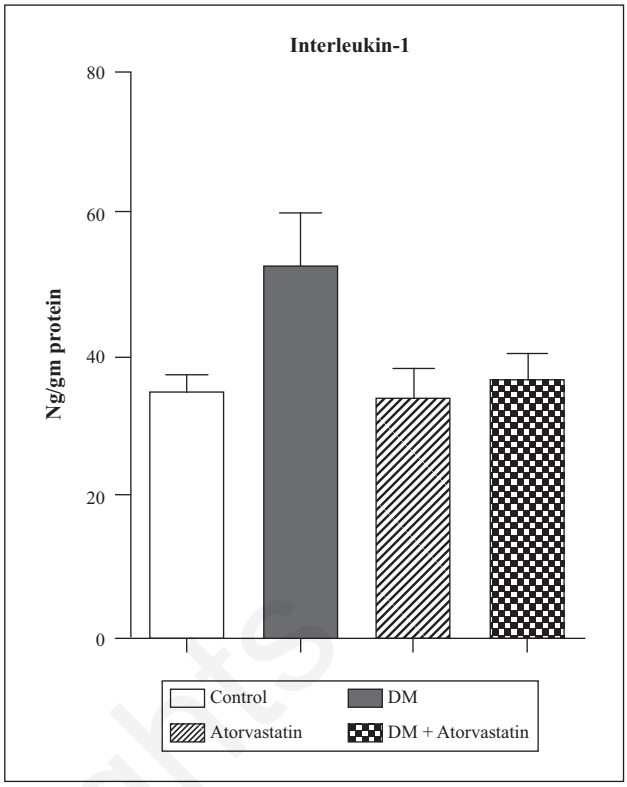

Figure 1
Effect of treatment with atorvastatin on the level of IL-1 β (mean ± SD) in the kidney of control and diabetic rats.

RESULTS

Changes in renal cytokines as a result of induction of type 1 diabetes and treatment with atorvastatin are presented in *table 1*. Induction of diabetes resulted in statistically significant elevation of all determined IL levels in kidney tissues. The pro-inflammatory IL-1 increased by 50.7% (*figure 1*) and IL-6 by 43.2% (*figure 2*), whereas the anti-inflammatory IL-10 increased by 41.2% (*figure 3*). An increase of 30.8% was detected for prostaglandin E2 (PGE2) (*figure 4*).

No statistically significant differences were detected between the levels of all the determined parameters in the naive animals and those treated with atorvastatin. However, following the treatment of diabetic rats, all the determined parameters tended to go back to levels comparable with those found in the control animals, with no statistically significant differences being detected (*table 1*). IL-1β

Table 1
Changes in the concentrations of ILs and PGE2 in the kidney of control and diabetic rats with and without treatment with atorvastatin.

	Normal	Diabetic	Atorvastatin	Diabetic + atorvastatin
IL-1β (ng/g protein)	34.9 ± 2.66	52.6 ± 7.59*	34.0 ± 4.16	36.6 ± 3.86#
IL-6 (ng/g protein)	76.8 ± 4.51	110 ± 19.5*	71.9 ± 8.76	78.8 ± 5.67#
IL-10 (ng/g protein)	41.0 ± 2.74	57.9 ± 12.7*	37.6 ± 4.87	44.5 ± 5.18#
PGE2 (ng/g protein)	1.17 ± 0.08	1.53 ± 0.12*	1.08 ± 0.13	1.22 ± 0.12#

* Significantly different from normal controls.
# Significantly different from diabetic rats.

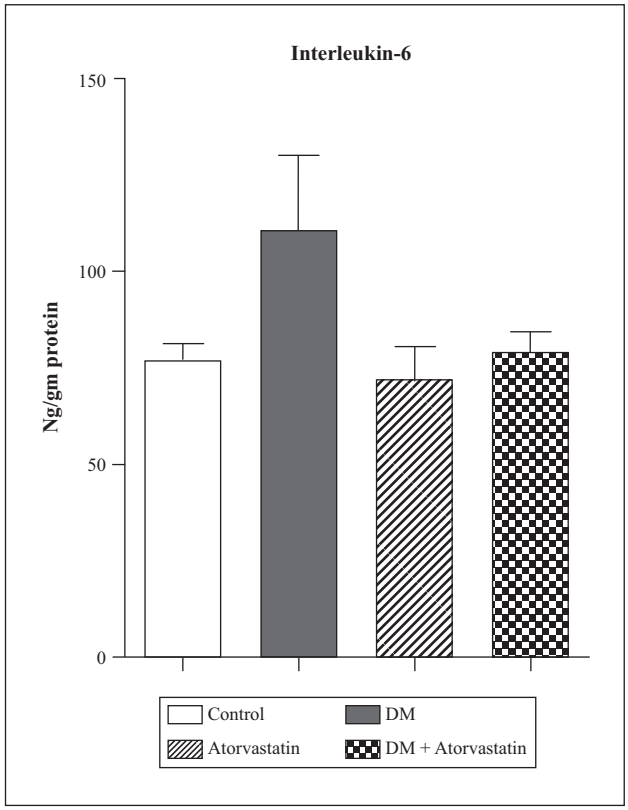

Figure 2
Effect of treatment with atorvastatin on the level of IL-6 (mean ± SD) in the kidney of control and diabetic rats.

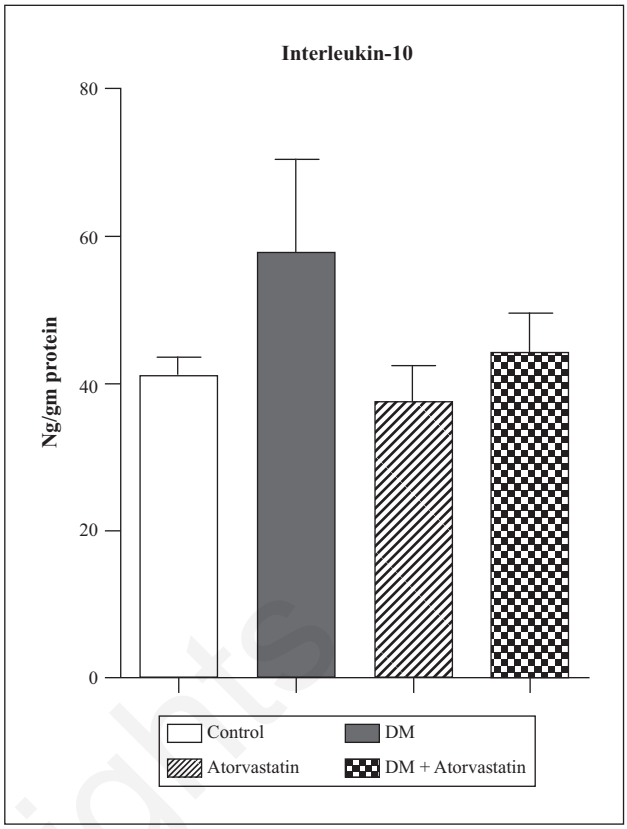

Figure 3
Effect of treatment with atorvastatin on the level of IL-10 (mean ± SD) in the kidney of control and diabetic rats.

decreased by 30.4% below the mean level of the diabetic animals (*figure 1*) and a decrease of about 28.4% was found with IL-6 (*figure 2*). The anti-inflammatory IL-10 decreased by 23.1% (*figure 3*). The level of PGE2 decreased by 20.3% below that of the diabetic animals (*figure 4*).

DISCUSSION

Cytokines act as pleiotropic polypeptides regulating inflammatory and immune responses through actions on cells. They provide important signals in the pathophysiology of a range of diseases, including diabetes mellitus. Chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of diabetes and its microvascular complications. Inflammatory cytokines, mainly IL-1, IL-6, and IL-18, as well as TNF-α, are involved in the development and progression of diabetic nephropathy. In this context, cytokine genetics is of special interest to combinatorial polymorphisms among cytokine genes, their functional variations, and general susceptibility to diabetic nephropathy. Finally, the recognition of these molecules as significant pathogenic mediators in diabetic nephropathy opens up the possibility of new potential therapeutic targets [24].

Cytokine measurements in tissue or in the peripheral circulation have been an important part of the process of defining the role that various cytokines play in health and disease. It has been suggested that local cytokine levels and activity are of considerably greater value for monitoring pathological events in a target tissue than systemic serum cytokine levels [25].

In the diabetic kidney, all the evaluated cytokines, IL-1β, IL-6, IL-10, and PGE2, were significantly elevated above the levels of the control group. It has been reported that some cytokines induce inflammation whereas others suppress inflammation. This concept is fundamental to cytokine biology and also to clinical medicine. Pro-inflammatory cytokine-mediated inflammation involves a cascade of gene products that are stimulated by IL-1, whereas anti-inflammatory cytokines, including IL-10, block or at least suppress the intensity of the cascade. It has been proposed that the biological activities of IL-10 in modulating inflammation may be caused, in part, by down-regulation of pro-inflammatory cytokines and the expression of their receptors and up-regulation of cytokine inhibitors [26]. Some studies have suggested that susceptibility to a disease is genetically determined by the balance between pro-inflammatory and anti-inflammatory cytokines [27].

Within a few minutes after binding to cells, IL-1 induces several biochemical events. One of the more universal activities of IL-1 is the induction of gene expression of cyclooxygenase-2 (COX-2). Once triggered, COX-2 production is elevated for several hours and large amounts of PGE2 are produced in cells stimulated with IL-1 [28]. This was observed in the present study by the concomitant elevation of PGE2 and IL-1. A broad spectrum of mediators regulates the expression of COX-2. Although pro-inflammatory cytokines such as IL-1β and IL-6, among other factors, induce COX-2, the anti-inflammatory cytokine IL-10 inhibits the expression of this enzyme [29]. PGE2 is one of the most abundant prostaglandins produced in the body and exhibits versatile biological activities.

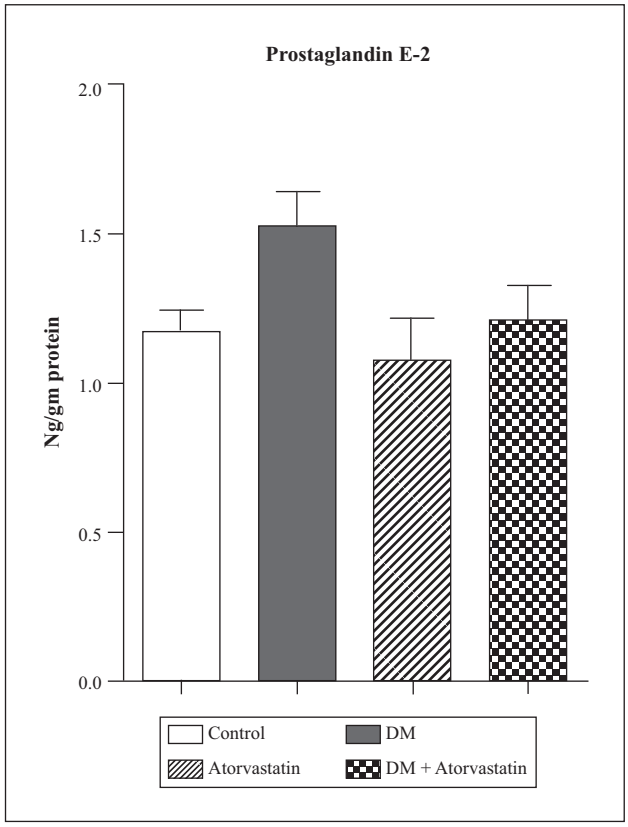

Figure 4
Effect of treatment with atorvastatin on the level of PGE2 (mean ± SD) in the kidney of control and diabetic rats.

Under physiological conditions, PGE2 is an important mediator of many biological functions including regulation of immune responses [30]. Dysregulated PGE2 synthesis or degradation has been associated with a wide range of pathological conditions [31]. It may, therefore, be involved in the mechanism of diabetes-induced renal injury, and the possible protective role of atorvastatin in this respect should be further elucidated.

In conclusion, despite the lack of clinical data, the results of the present study suggest that atorvastatin has the potential to protect or attenuate diabetes-induced renal injury through preventing elevation of proinflammatory cytokines levels and maintaining them at near-control values.

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