Iranian Journal of Pharmaceutical Research (2006) 3: 191-198
Received: May 2005
Accepted: November 2005

Original Article

Lovastatin Incubation Improves Acetylcholine-Induced Relaxation in Isolated Aortic Rings of Diabetic Rat

Heydar Parsaee*, Mohsen Imenshahidi and Zahra Fatehi

Department of Pharmacology, Department of Physiology, Pharmacological Research center of Medicinal plants Medical school, Mashhad University of Medical sciences.

Abstract

To evaluate the acute effect of lovastatin on diabetic endothelial dysfunction, we examined this effect on the aortic rings of streptozotocin-diabetic rats. The endothelial function was assessed in aortic rings isolated from diabetic rats, 12 weeks after treatment with streptozotocin (45 mg/kg, i.p.). The concentration-response curve to acetylcholine (Ach) in the aortic rings precontracted with phenylephrine (10^{-6} M) was significantly diminished in diabetic groups; and maximum relaxation in control and diabetic groups were 82±1.93% and 48±2.39% respectively (a 42% decrease, $P<0.001$). Incubation with lovastatin (10^{-5} M) for 10 min, significantly improved the Ach-induced relaxation of diabetic groups and the maximum relaxation increased to 74.2 ±3.3% (a 54% increase, $P<0.001$).

Incubation with N^G –nitro-L-arginine methyl ester hydrochloride (L-NAME; $5x10^{-7}$ M) for 20 min eliminated a significant difference in Ach – induced relaxation responses in diabetic and control groups and also eliminated the improving effect of lovastatin in diabetic groups. On the other hand 10 min incubation with indomethacin (10^{-5} M) did not eliminate the difference in Ach-induced relaxation responses in diabetic and control groups and also did not eliminate the improving effect of lovastatin in diabetic groups. Lovastatin did not modify sodium nitroprosside-induced relaxation in either diabetic or control groups and also did not induce any direct relaxation.

Therefore, it is concluded that incubation of aortic rings with lovastatin significantly improves endothelium-dependent relaxation in diabetic groups by increasing the nitric oxide bioavailability, most probably due to its' antioxidant effects.

Keywords: Endothelium; Streptozotocin; Diabetes; Rat aortic rings; Lovastatin; Antioxidant.

Introduction

Macro- and microvascular diseases are currently the principal causes of morbidity and mortality in patients with type I and type II diabetes mellitus. The pathophysiological processes underlying vascular complications are still poorly understood. However, in recent years, several lines of evidence have suggested that some of the vascular changes in diabetes may be related to alterations in endothelial function (1). Under normal conditions, the endothelium contributes to the regulation of vascular reactivity through the release of different relaxants [nitric oxide (NO), prostacyclin (PGI_2), and endothelium-derived hyperpolarising factor (EDHF)] or constrictors (angiotensin II, endothelin-1, thromboxane A_2,

* Corresponding author:
E-mail: h-parsaee@mums.ac.ir.

and prostaglandin H_2), the equilibrium of which results in vascular tone (2). But in diabetes, loss of the modulatory role of the endothelium may be a critircal and initiating factor in the development of diabetic vascular disease (1). During endothelial dysfunction; which is induced in arterial hypertension, hypercholesterolemia, postmenopause and diabetes (3); responsiveness to endothelium-dependent vasodilators such as acetylcholine is reduced. Several mechanisms have been suggested for this abnormality including: activation of diacylglycerol-protein kinase C, formation of advanced glycation end products (AGEs) and an increased oxidative stress, activation of polyol pathway and a reduced bioavailability of NO. Different causes could reduce NO bioavailability, such as abnormalities in signal transduction, reduced synthesis of NO and an enhanced inactivation of NO (3).

The endothelial dysfunction promotes atherosclerosis by mechanisms which is not fully underestood (4). Therefore, recent interest has focused on strategies to reverse or prevent the enthothelial dysfunction in order to modify the natural history of diabetic vascular disease (1). In this regard, pharmacological intervention remains a reasonable, less expensive and potentially safer option to protect or restore diabetic endothelial dysfunction (5).

One class of drugs, that is under investigation for this purpose, is statins. Statins, as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, have been described as the most effective class of drugs used to reduce serum cholesterol levels, and have been shown to significantly reduce cardiovascular events and mortality in patients with or without coronary artery disease (6). All beneficial effects of statins can not be explained by their effects on plasma lipids. Statins exert many pleiotropic effects (vascular effects unrelated to lipid) on the vascular wall, including beneficial effect on endothelial function and blood flow, decreasing LDL oxidation, enhancing the stability of atherosclerotic plaques, inhibiting vascular smooth muscle prolifration and migration, platelet aggregation and reducing vascular inflamation.

Regarding the effect of statins effect on diabetes-induced endothelial dysfunction, there are fewer studies and their results are controversial. Administration of pravastatin (10 mg/kg for 4 weeks) to streptozotocin-induced diabetic rats preserves endothelial function in aorta, without lowering plasma cholesterol and this effect has been found responsible for decreasing LDL oxidation (7). In another study pravastatin has restored endothelial function in carotid artery of insulin resistant rats and this beneficial effect has been ascribed to the augmentation of NO production (8). In a clinical study, short term simvastatin treatment had no effect on lipid parameters, but showed a beneficial effect on oxidative stress and endothelial dysfunction (9).

Conversely, in some studies adminisration of statins has not been found effective on endothelial dysfunction. Administration of simvastatin (40 mg for 6 weeks) did not change the endothelium dysfunction in diabetic patients (10). Also in streptozotocin-induced diabetic rats, simvastatin did not improve the abnormal relaxation to acetylcholine (11).

To further evaluate the direct effect of statins on diabetic endothelial dysfunction, in the present study, we investigated the effect of lovastatin on the aortic rings of streptozotocin-induced diabetic rats.

Experimental

Animals

The experiments were performed in accordance to the animals Act of 1986 (Britain) and they conformed to the National Institutes of Health guidelines for the use of experimental animals in Britain (The study was approved by the ethical committee of Mashhad University of Medical Sciences). Diabetes was induced in male wistar rats (Razi Institutes, Mashhad, Iran), weighing 200-250 g, by a single intraperitoneal injection of streptozotocin (45 mg/kg) dissolved in sterile 0.9% NaCl solution (12). Non-diabetic control animals were injected with an equivalent volume of vehicle. Both groups were maintained under the same conditions, supplied with food and water ad libitum until death. Manifestation of diabetes was verified one week later by blood sampling from the tail vein and determination of glucose by glucometer and test strips (Lifescan, Inc, USA).

Rats were considered as diabetic when blood glucose levels were more than 270 mg/dl (13).

Tissue preparation

After 12 weeks of diabetes induction, rats were anesthetized with interaperitoneal injection of sodium thiopental (80 mg/kg). Then animals were killed by opening the thorax and cardiectomy. The descending thoracic aorta was rapidly dissected and placed into ice-cold Krebs-Henseleit solution (KHS), with a composition (in mM) of: NaCl 118, KCl 4.7, $MgSO_4$ 2 H_2O 1.2 KH_2PO_4, 2 H_2O 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 11.1. The aorta was separated from the surronding connective tissue and cut into rings (4 mm).

Special care was taken not to damage the endothelium. The rings were then suspended in organ chambers, between a clip and a force-displacement transducer under a resting tension of 1.5 g (in preliminary studies deteminerd to be optimum), in order to measure the isometric force and recording it on a George Washington oscillograph (400 MD/2).The organ chamber was filled with 25 ml of KHS at 37° C and gassed with 95% O_2+5% CO_2. Aortic rings were challenged with 1 µM phenylephrine (PE) and then allowed to equilibrate for 1 h. During this equilibrium period the physiological salt solution was replaced every 15 min.

Concentration-response curve for PE (10^{-8}-10^{-4} M) was obtained by cumulative addition of PE to the bath solution. The endothelium dependent were relaxations recorded in PE (10^{-6} M) preconstricted rings for acetylcholine alone, and after 20 min incubation with N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME, $5x10^{-7}$ M) to block the NO synthase (14). Endothelium dependent relaxations also were recorded after 10 min incubation with lovastatin (10^{-5} M) alone and 20 min incubation with L-NAME ($5x10^{-7}$ M) plus 10 min incubation with lovastatin (10^{-5} M).

To examine the role of cyclooxygenase pathway, relaxation induced by cumulative concentrations of Ach were determined after 10 min preincubation with indomethacin (10^{-5}M) and 10 min preincubation with indomethacin (10^{-5} M) plus lovastatin (10^{-5} M). Endothelium-independent vasorelaxation to NO donor sodium nitroprusside (SNP, 10^{-9}, $5x10^{-9}$ and 10^{-8}M) alone and after 10 min incubation with lovastatin (10^{-5}M) were also assessed.

To examine the possible direct relaxation induced by lovastatin, the precontracted rings with PE (10^{-6} M) were incubated with lovastatin for 10 min (10^{-5} and 10^{-4} M) and 30 min (10^{-5}M) and changes in tension were recorded.

The endothelium was removed by gentle rubbing of the aortic ring between thumb and forefinger, and lack of responsiveness to Ach confirmed endothelium denudation (Hopfner, 1999). In order to determine the effect of lovastatin in the absence of endothelium, the endothelium-denuded and precontracted rings with PE (10^{-6} M), were incubated with lovastatin for 10 min (10^{-5} and 10^{-4}M) and changes in tension were recorded.

All the above experiments were performed with aortic rings isolated from control and diabetic animals.

Drugs

Acetylcholine chloride, phenylephrine hydrochloride, L-NAME, SNP and indomethcine were obtained from Sigma. Sodium chloride, potassium chloride, magnesium sulfate, sodium hydrogen carbonate, potassium hydrogen orthophophate, D-glucose, hydrochloric acid, sodium chloride and calcium chloride were obtained from Merk laboratories. Sodium thiopental was obtained from Biochemie GmbH (Vienna, Austria). Streptozotocin was obtained from Pharmacia & Upjohn (USA). Lovastatin was a generous gift from Dr Abidi Laboratories (Tehran, Iran). Lovastatin in parent lacton-form is inactive (6), and was converted to the active form (β-hydroxy acid) by dissolving 20 mg of the lacton-form in 0.5 ml of ethanol (95%), adding 0.75 ml of NaOH (0.1 M) and heated at 50° C for 2 h, then neutralized with HCl (0.1 M) to a pH of approximately 7.2 and adjusted with distilled water to a volume of 10 ml. This stock-solution (5 mM) was stored frozen and diluted to the working concentration with distilled water (15).

Data analysis

Results are expressed as mean ± SEM, and analyzed by two way analysis of variance

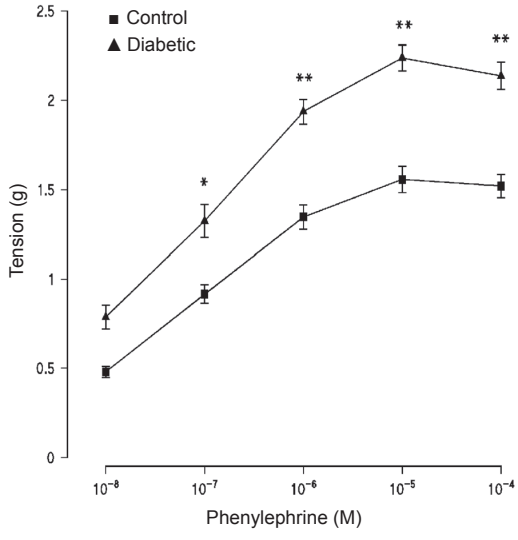

Figure 1. Concentration-response curves showing vasoconstrictor responses of rat aortic rings to phenylephrine; control (■), diabetic (▲). Values are expressed as mean ± S.E.M. from 8-10 animals. * P<0.05 and ** P< 0.01 diabetic vs. control.

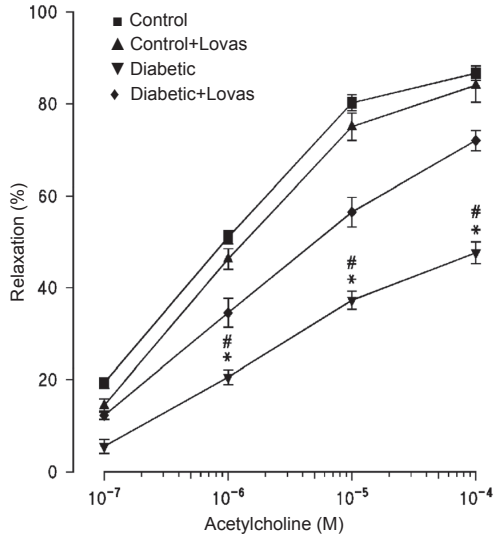

Figure 2. Concentration-response curves showing acetylcholine-induced relaxation in phenylephrine-precontracted (10^{-6}M) rat aortic rings. Relaxation is expressed as the percentage of reduction in the phenylephrine-induced increase in tone; control (■), diabetic (▼), control incubated 10 min with lovastatin (10^{-5}M) (▲), diabetic incubated 10 min with lovastatin (10^{-5}M) (♦). Values are expressed as mean ± S.E.M. from 8-10 animals. * P<0.001 diabetic vs. control, # P< 0.001 diabetic vs. diabetic + lovastatin.

(ANOVA) followed by Tukey-Kramer multiple comparison test. A p-value less than 0.05 was considered as significant.

Results and Discussion

Blood glucose levels

Blood glucose concentrations are given in Table 1. Diabetes caused an approximately 3 fold elevation in blood glucose (P<0.001). Distention of the large and small intestines and the presence of a pale watery stool were observed in killed diabetic animals.

Vasoconstrictor responses to phenylephrine (PE)

Vasoconstrictor responses to different concentrations of PE (10^{-8}M - 10^{-4}M) in the aortic rings of diabetic rats were significantly increased and as shown in Figure 1, the maximum responses in control and diabetic groups are 1.56±0.074 and 2.23±0.072 g tension respectively (a 43% increase, n=8, p<0.001).

Vasodilator responses to acetylcholine and sodium nitroprusside

As shown in Figure 2, the concentration-dependent relaxation responses to Ach in the aortic rings precontracted with PE (10^{-6}M) have significantly diminished in diabetic groups. Maximum relaxation responses in control and diabetic groups were 82±1.93 (n=10) and 48±2.39% (n=8) respectively (a 42% decrease, p<0.001). Incubation with lovastatin (10^{-5}M) for 10 min significantly improved the Ach-mediated relaxation in diabetic groups (P<0.001), and the maximum relaxation increased to 74.2±3.3% (a 54% increase), although could not restore to the control levels. Lovastatin did not significantly change the Ach-induced relaxation in the control group (P>0.05).

Incubation with nitric oxide inhibitor, L-NAME ($5x10^{-7}$M), for 20 min eliminated the significant difference in Ach-induced relaxation response between the diabetic and control groups (P>0.05). Thus, the concentration-response curves of the control and diabetic groups became superimposed. Also incubation with lovastatin

Table 1. Blood glucose concentrations (mg/dl) in control and diabetic rats, 2 and 12 weeks after treatment with either saline or streptozotocin. Data are presented as mean ± S.E.M, * P< 0.001.

Groups	2 weeks	12 weeks
Control (n=10)	156 ± 15	158 ± 19
Diabetic (n=8)	464 ± 30*	442 ± 31*

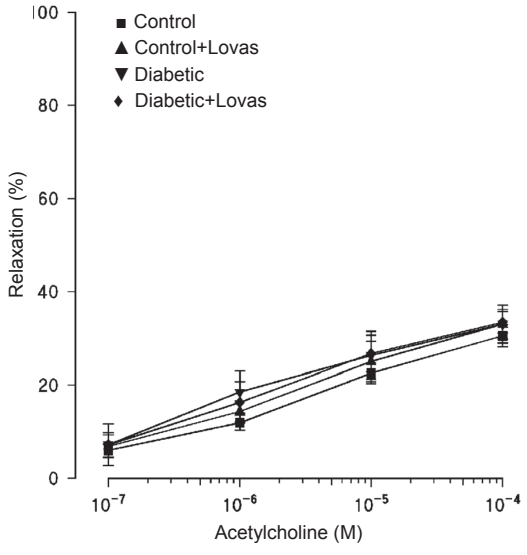

Figure 3. Concentration-response curves showing acetylcholine-induced relaxation in phenylephrine-precontracted (10^{-6}M) rat aortic rings after preincubation with L-NAME ($5x10^{-7}$M) for 20 min. Relaxation is expressed as the percentage of reduction in the phenylephrine-induced increase in tone; control (■), diabetic (▼), control incubated 10 min with lovastatin (10^{-5}M) (▲), diabetic incubated 10 min with lovastatin (10^{-5}M) (♦). Values are expressed as mean ± S.E.M. from 8-10 animals. There is no significant difference between the groups (P> 0.05).

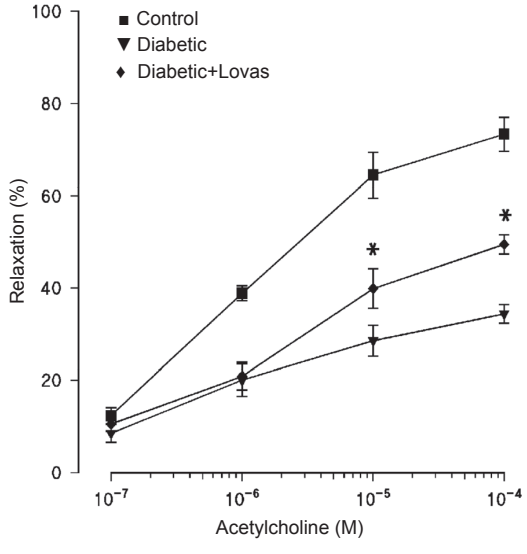

Figure 4. Concentration-response curves showing acetylcholine-induced relaxation in phenylephrine-precontracted (10^{-6}M) rat aortic rings after preincubation with indomethacin (10^{-5}M) for 10 min. Relaxation is expressed as the percentage of reduction in the phenylephrine-induced increase in tone; control (■), diabetic (▼), diabetic incubated 10 min with lovastatin (10^{-5}M) (♦). Values are expressed as mean ± S.E.M. from 8-10 animals. * P< 0.05 diabetic vs. diabetic + lovastatin.

could not improve the relaxation responses to Ach, when the aortic rings preincubated with LNAME in either diabetic or control groups (Figure 3).

On the other hand, 10 min incubation with the cyclo-oxygenase inhibitor, indomethacin (10^{-5}M), did not eliminate the difference in Ach-induced relaxation responses between the diabetic and control groups and the maximum relaxation responses were 73.3±3.7% and 34.3±2% respectively ($p<0.001$). Incubation with lovastatin could significantly improve the relaxation responses to Ach in diabetic groups in the presence of indomethacin and the maximum relaxation increased to 49.5 ±2.1% (44% improvement, Figure 4).

Endothelium independent vasorelaxation to different concentrations of SNP (10^{-9} -10^{-8}M) was unimpaired in diabetic groups and maximum relaxations were not significantly different in the control and diabetic groups, either in the presence or absence of lovastatin (P>0.05, Figure 5). Therefore, lovastatin-treatment did not change the responsiveness of diabetic and control aortic rings to SNP.

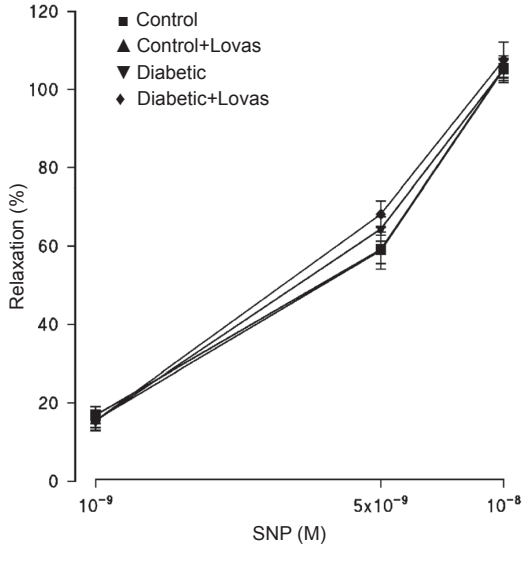

Figure 5. Concentration-response curves showing vasodilator responses of rat aortic rings to sodium nitroprusside precontracted with phenylephrine (10^{-6}M). Relaxation is expressed as the percentage of reduction in the phenylephrine-induced increase in tone; control (■), diabetic (▼), control incubated 10 min with lovastatin (10^{-5}M) (▲), diabetic incubated 10 min with lovastatin (10^{-5}M) (♦). Values are expressed as mean ± S.E.M. from 8-10 animals. There is no significant difference between the groups (P> 0.05).

Direct effect of lovastatin on aortic rings

Incubation of PE (10^{-6}M) precontracted aortic rings with different concentrations of lovastatin (10^{-5}-10^{-3}M) alone did not cause any significant change in the tension of control and diabetic groups (Figure 6). Identical results were observed, when compating endothelium-intact and endothelium-denuded aortic rings from control and diabetic groups (data not shown).

The results of this study show that a 12 weeks diabetes period significantly reduce endothelium-dependent relaxation of aortic rings to acetylcholine (a 42% reduction) and 10 min incubation of aortic rings with lovastatin significantly improved endothelium-dependent relaxation (a 54% improvement). On the other hand, lovastatin alone could not induce direct vasorelaxation in precontracted aortic rings taken from control or diabetic animals.

Pre-incubation of control and diabetic aortic rings with L-NAME eliminated the difference in Ach-induced relaxations in these two groups, indicating that endothelial dysfunction in diabetic animals is related to NO pathway (Figure 3). These results are consistent with other works (14, 16).

Pre-incubation of control and diabetic aortic rings with indomethacin could not diminish the improving effect of lovastatin on Ach-induced relaxation in diabetic groups, suggesting that cyclooxygenase pathway has no significant role in endothelial dysfunction in diabetic animals and further confirms the role of NO pathway (Figure 4).

The reduction of acetylcholine-induced relaxation in diabetic groups is not due to a decrease in the sensitivity of vascular smooth muscle to NO, since responses to SNP were similar in diabetic and control groups, which is in agreement with the previous studies (13, 16). The improving effect of lovastatin on acetylcholine-induced relaxation in diabetic groups is not mediated by an increase in the sensitivity of vascular smooth muscle to NO, since responses to SNP were not different in diabetic and control groups (Figure5).

Therefore, based on the above findings, it is concluded that the improving effect of lovastatin on endothelial dysfunction is mainly related to NO bioavailability.

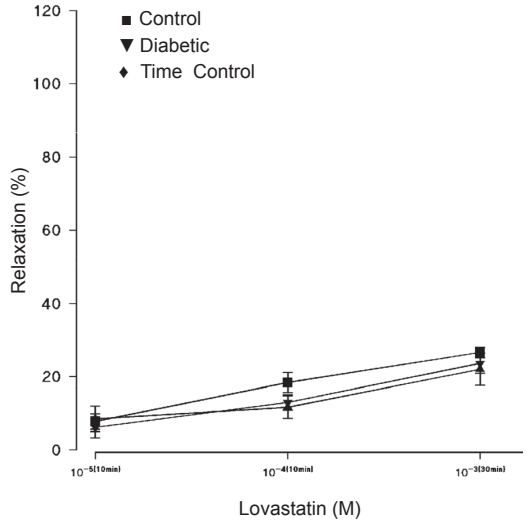

Figure 6. Concentration-response curves showing the effect of lovastatin incubation on phenylephrine-precontracted (10^{-6}M) rat aortic rings. Relaxation is expressed as the percentage of reduction in the phenylephrine-induced increase in tone; control (■), diabetic (▲), time control (▼) (this group has been used to determine autorelaxation which occurs in aortic rings after 10, 20 and 40 min incubation with krebs solution). Values are expressed as mean ± S.E.M. from 8-10 animals. There is no significant difference between the three groups (P> 0.05).

Two different pathways could contribute to the net reduction of bioavalable NO in diabetes, including the reduced synthesis of NO which has been reported in some studies (17, 16) but in others (5); and more probably enhanced inactivation of NO due to free radicals. Diabetes has been associated with an increased generation of oxygen-drived free radicals (4). Sources of reactive oxygen species in diabetes may include autooxidation of glucose (4), advanced glycation end-products (AGE) formation and the binding of AGE5 to their receptors (13, 18) and an increased substrate flux through the polyol pathway (4).

Oxygen-derived free radicals impair endothelium-dependent vasodilation through inactivation of NO or by serving as an endothelium-derived constricting factor (13;, 16, 19 and 20). Acute administration of superoxide anion scavenger, superoxide dismutase, improved or normalized the abnormal endothelium-dependent responses in different models of diabetes and during a high glucose exposure (1). Similarly, chronic treatment with probucol (21), vitamin E (13, 22) and vitamin C (23) prevented the development of endothelial dysfunction in

clinical and experimental diabetes.

It seems that among these two probable mechanisms for decreasing NO bioavalability, antioxidant effect is involved in the present study because lovastatin alone has not induced relaxation. If it had stimulated endothelial NO synthase, it should have induced relaxation alone, as has been shown in another work with pravastatin under hypercholesterolemic condition (24). On the other hand, antioxidant activity of statins in chronic and acute administration have been shown in several studies (25- 27).

Collectively, lovastatin improves aortic endothelial dysfunction in the streptozotozocin-diabetic model, probably via its antioxidant effect. However, such an acute treatment does not normalise NO-mediated relaxation, perhaps because it can not compensate for endothelial damage that has progressed to irreversible injury.

Acknowledgment

This study was supported by a grant from the research council of Mashhad University of Medical Sciences.

References

(1) De Vriese A, Verbeuren TJ, Voorde JV, Lameire NH, Vanhoutte PM (2000): Endothelial dysfunction in diabetes. *Br J Pharmacol* 130: 963-74.

(2) Miranda FJ, Alabodi JA, Leorens S, Ruiz de Apodaca RF, Centeno JM, Alborch E (2000): Diabetes-induced changes in endothelial mechanisms implicated in rabbit carotid arterial response to 5-hydroxytryptamine. Eur J Pharmacol 401: 397-402.

(3) Hayoz D, Ziegler T, Brunner HR, Ruiz J (1998): Diabetes mellitus and vascular lesion. *Metabolism* 47: 16-19.

(4) Giugliano D, Ceriello A (1996): Oxidative stress and diabetic vascular complications. *Diabetes Care* 19: 257-267.

(5) Pieper GM, Siebeneich W, Olds CL, Felix CC, Soldato PD (2002): Vascular protective actions of a nitric oxide aspirin analog in both in vitro and in vivo models of diabetes mellitus. *Free radical Biology & Medicine* 32: 1143-56.

(6) Veillard NR, March F (2002): Statins; the new aspirin? *Cell Mol Life Sci* 59: 1771-86.

(7) Kobayashi T, Matsumoto T, Kumata K (2000): Mechanisms underlying the chronic pravastatin treatment-induced improvement in the impaired endothelium-dependent aortic relaxation seen in streptozotocin-induced diabetic rats. *Br J Pharmacol* 131: 231-8.

(8) Dumont AS, Hyndman ME, Dumont RJ, Fedak PM, Kassell NF, Sutherland GR, Verma S (2001): Improvement of endothelial function in insulin-resistant carotid arteries treated with pravastatin. *J Neurosurgery* 95: 466-71.

(9) Ceriello A (2002): Nitrotyrosine new findings as a marker of postprandial oxidative stress. Int. *J Clin Pract Suppl* 129: 51-8.

(10) Van de Ree MA, Huisman MV, De man FH, Van der Vigver HC, Meinders AE, Blauw GJ (2001): Impaired endothelium-dependent vasodilation in type 2 diabetes mellitus and the lack of eefct of simvastatin. *Cardiovascular Res* 52: 299-305.

(11) Palmer AM, Gopaul N, Dhir S, Thomas CR, Poston L, Tribe RM (1998): Endothelial dysfunction in streptozotocin-diabetic rats is not reversed by dietary probucol or simvastatin supplementation. *Diabetologia* 41: 157-64.

(12) Cotter MA, Jack AM, Cameron NE (2002): Effects of the protein kinase C inhibitor LY333531 on neural and vascular function in rats with streptozotocin-induced diabetes. *Clin Sci* 103: 311-21.

(13) Rosen P, Ballhausen T, Stockklauser K (1996): Impaired of endothelium dependent relaxation in the diabetic rat heart: mechanisms and implications. *Diabetes Res Clin Practice* 31: 143-155.

(14) Kazuki E, Abiru T, Machida H, Kasaya Y, Kamata K (1995): Endothelium derived hyperpolarizing factor dose not contribute to the decrease in endothelium-dependent relaxation in the aorta of streptozocin-induced diabetic rats. *Gen Pharmacol* 26: 146-153.

(15) Schmidt A, Goepfert C, Feisma K, Buddecke E (2002): Lovastatin-stimulated superinduction of E-selectin, ICAM-1 and VCAM-1 in TNF- activated human vascular endothelial cells. *Atherosclerosis* 164: 57-64.

(16) Pieper GM (1998): Review of alterations in endothelial nitric oxide production in diabetes: Protective role of arginine on endothelial dysfunction. *Hypertension* 31: 1047-1060.

(17) Taylor PD, Graves JE, Doston L (1995): Selective impairment of acetylcholine mediuted endothelium-dependent relaxation in isolated resistance arteries of the streptozocin-induced diabetic rat. *Clin Sci* 88: 519-24.

(18) Taniguchi NH, Asahi M, Takahashi M (1996): Involvement of glycation and oxidative stress in diabetic macroangiopathy. *Diabetes* 45: s81-s38.

(19) Graier WF, Posch K, Fleischhacker E, Wascher TC, Kostner G M (1999): Increased superoxide anion formation in endothelial cells during hyperglycemia: an adoptive response or initial step of vascular dysfunction? *Diabetes Res And Clin Practice* 45: 153-160.

(20) Laight DW, Carrier MJ, Anggard EE (2000): Antioxidants, diabetes and endothelial dysfunction. *Cardiovoscular Res* 47: 457-64.

(21) Tesfaramiam B, Cohen RA (1992): Free radical mediate endothelial cell dysfunction caused by elevated glucose. *Am J Physiol* 263: H321-326.
(22) Keegan A, Walbank H, Cotter MA, Cameron NE (1995): Chronic vitamine E treatment prevent defective endothelium dependent relaxation in diabetic rat aorta. *Diabetologia* 38: 1475-78.
(23) Ting HH, Timimie FK, Boles KS (1996): Vitamine C improves endothelium-dependent vasodilation in patient with non-insulin dependent diabetes mellitus. *J Clin Invest* 97: 22-28.
(24) Kaesmeyer WH, Caldwell RB, Huang J, Caldwell RW (1999): Pravastatin sodium activates endothelial nitric oxide synthase independent of it's Cholesterol-lowering actions. *J Am College of Cardiology* 33: 234-41.
(25) Mueck AO, Seeger D, Wallwiener D (2001): Further evidence for direct vascular actions of ststins: Effect on endothelial nitric oxide synthase and adhesion molecules. *Exp Clin Endocrinol Diabetes* 109: 181-183.
(26) Vaughan CJ, Delanty N (1999): Neuroprotective properites of statins in cerebral ischemia and stroke. *Stroke* 30: 1969-1973.
(27) Aviram M, Rosenblat M, Bisgaier VL, Newton RS (1998): Atorvatatin and gemfibrozil metabolites, but not the parent drugs are potent antioxidants against lipoprotein oxidation, *Atherosclerosis* 138: 1271-1280.

This article is available online at http://www.ijpr-online.com