EXTENDED REPORT

Effect of atorvastatin on ocular blood flow velocities in patients with diabetic retinopathy

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Aim: To investigate blood flow velocities in the ophthalmic and central retinal arteries (CRAs) in patients with diabetic retinopathy before and after atorvastatin treatment.

Methods: 45 patients with type 2 diabetes were included in this double-blind, placebo-controlled study. The patients with diabetes were divided into three subgroups: group 1 (n = 15) included patients with non-proliferative diabetic retinopathy (NPDR); group 2 (n = 15) had patients with proliferative diabetic retinopathy (PDR); and group 3 (n = 15; placebo group) included 8 patients with NPDR and 7 patients with PDR. The patients in groups 1 and 2 (atorvastatin group) received 10 mg atorvastatin daily for 10 weeks. Pre-treatment and post-treatment serum levels of total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride were recorded before and after treatment. Ocular blood flow velocities of the ophthalmic artery and CRA were evaluated by colour Doppler imaging before and after treatment in each group.

Results: The baseline haemodynamic parameters were similar between atorvastatin and placebo groups ($p>0.05$ for both). Atorvastatin significantly decreased serum levels of total cholesterol, low-density lipoprotein cholesterol and triglycerides in groups 1 and 2 compared with pretreatment levels ($p<0.001$ for both). The mean peak systolic flow velocities (PSVs) of the ophthalmic artery in group 2, and the mean PSV and resistive indices of the CRA in groups 1 and 2 decreased significantly after atorvastatin treatment ($p<0.05$ for both), whereas the mean end diastolic flow velocity of the ophthalmic artery and CRA did not change ($p>0.05$). There was no significant difference in ocular blood flow velocities in the placebo group ($p>0.05$).

Conclusion: Atorvastatin may have a role in reducing diabetic retinal complications, with improvement in vascular resistance and decrease in the mean PSVs of the ophthalmic artery and CRA. However, further studies with large numbers of patients are needed to obtain the long-term results of this drug.

Diabetes mellitus is associated with systemic and ocular microvascular abnormalities, but the mechanism behind it is not yet clearly understood. The two main abnormalities in diabetic retinopathy are increased retinal vascular permeability and progressive retinal vascular occlusion, which lead to tissue hypoxia and ischaemia with neovascularisation of the retina by angiogenetic factors.[1–3] Although the effect of diabetes on the ocular circulation is poorly understood, altered retinal circulation is well documented in eyes with diabetic retinopathy.[4–8] The normal endothelium has a key role in the local regulation of the vascular tone by producing and releasing both contracting and relaxing factors.[9] Endothelial dysfunction with increased generation of oxygen-derived free radicals was shown in animal models of type 1 diabetes mellitus[10 11] and in young patients with diabetes.[12–14] Major functional consequences of endothelial dysfunction in diabetes are reduced bioavailability of endothelium-derived nitric oxide with impaired endothelial-dependent dilatation in either type of human diabetes.[14 15]

Recently, improvement in endothelial function was shown after various treatments such as lipid-lowering drugs,[16] antioxidants,[17] angiotensin-converting enzyme inhibitors,[18] calcium channel blockers[19] and oestrogen replacement.[20] Inhibitors of hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase (statins) have currently been used extensively to lower serum cholesterol levels with their proved antithrombotic, antiproliferative and anti-inflammatory properties.[21 22]

Atorvastatin, a new HMG-CoA reductase inhibitor, was shown to be effective in preventing oxidation of low-density lipoprotein, which impairs endothelial-dependent dilatation.[23] Simons *et al*[24] showed that atorvastatin improves blood flow in the forearm, causing reactive hyperaemia. However, no data are currently available about the effects of atorvastatin on ocular blood flow. Because statins directly decrease the expression of endothelin 1 and increase the activity of endothelial nitric oxide synthase with improved endothelial function, we hypothesised that atorvastatin treatment has beneficial and potentially synergistic effects on endothelial function and also on ocular blood flow in patients with diabetes. Therefore, in this study, we measured blood flow velocities in the ophthalmic and central retinal arteries (CRAs) of patients with type 2 diabetes before and after atorvastatin treatment.

PATIENTS AND METHODS

45 patients with type 2 diabetes were included in this study. The study was carried out according to the Helsinki Declaration and good clinical practice regulations, and approved by the ethics review committee of the University Hospital, Erciyes University, Kayseri, Turkey. All participants gave their informed consent.

Patients with diabetes

All patients from similar ethnic backgrounds were recruited from the clinical practice of the Retina Service at the Medical Faculty, Erciyes University, Kayseri, Turkey. All patients were

Abbreviations: CDI, colour Doppler imaging; CRA, central retinal artery; EDV, end diastolic velocity; HMG-CoA, hydroxymethyl glutaryl coenzyme A; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; PSV, peak systolic velocity

previously diagnosed with type 2 diabetes according to the criteria of the American Diabetic Association.

Patients were excluded if any of the following were present: type 1 diabetes, primary or secondary hypertension, heart failure, peripheral vascular disease, severe renal dysfunction, nephrotic syndrome or dysproteinaemias, cigarette smoking, vasoactive drugs, antioxidant compounds, hormone replacement therapy, fasting plasma triglycerides >200 mg/dl, aspartate aminotransferase or alanine aminotransferase levels >1.5 times the upper limit of normal, or creatine phosphokinase levels >3 times upper limit of normal.

Because some drugs (eg, corticosteroids, cytotoxics, non-steroidal agents) could affect the levels of nitric oxide indirectly by affecting the function of cells that make nitric oxide, a detailed history of medication was obtained in both groups, and such patients were excluded. At inclusion, glycated haemoglobin had to be <10%, body mass index <35 kg/m^2, and at two occasions within 2 months before start of the study, fasting total cholesterol had to be <220 mg/dl.

The levels of diabetic retinopathy were determined by fundus findings according to the Early Treatment of Diabetic Retinopathy Study. All participants underwent a complete ophthalmic examination consisting of history, visual acuity, slit-lamp biomicroscopy, detailed fundus examination with a 90-dioptre lens, and fundus fluorescein angiography.

Study protocol

The protocol was designed as a double-blind placebo-controlled study. Blood samples were taken, and ocular blood flow was evaluated by colour Doppler imaging (CDI) in patients with diabetes. After initial examination, patients with type 2 diabetes were classified as having (1) non-proliferative diabetic retinopathy (NPDR; n = 23) or (2) proliferative diabetic retinopathy (PDR; n = 22). The first 15 patients in each classification then received atorvastatin (10 mg/day) for a period of 10 weeks and the remainder received placebo (group 3 (n = 15 NPDR, n = 8; PDR, n = 7). Thus, group 1 consisted of patients with NPDR who received atorvastatin (n = 15) and group 2 consisted of patients with PDR who received atorvastatin (n = 15). The drugs were taken in the morning except on the examination days to eliminate their acute effect. All participants were instructed not to change their dietary and drinking habits, or the regimen for diabetes treatment during the whole study. They were also warned not to take any additional drug including vitamin supplements, and were asked to come regularly once in 15 days to check the compliance with the treatment regimen. At the end of this period, a physical examination was performed; blood samples were taken and CDI examination was completed.

Biochemistry

All blood samples were collected in the morning hours (08:30–09:30) with the patient in the supine position after an overnight fasting, and samples were immediately processed. Serum levels of total cholesterol, high-density lipoprotein cholesterol and triglycerides were enzymatically determined using an oto-analyser (Kone 60I) and the same commercially available kits (Konelab, Thermo Clinical Labsystems, Vantaa, Finland). Low-density lipoprotein cholesterol was calculated according to the Friedewald's formula and non-high-density lipoprotein cholesterol as the difference between total cholesterol and high-density lipoprotein cholesterol.

Colour Doppler imaging

The left eye was studied in all patients to assess blood flow velocity of the ophthalmic artery and the CRA. All CDI examinations were performed with the patient in the supine position by the same experienced sonographer using a PowerVision 6000 analyser (Toshiba, Kyoto, Japan) with a 7.5-MHz linear transducer in a blinded manner. The transducer was applied gently to the closed eye using a coupling gel. Care was taken to avoid applying any excessive pressure on the eye.

Peak systolic velocity (PSV; the highest velocity achieved during a systole), end diastolic velocity (EDV; the lowest velocity achieved during a diastole) and resistivity index (RI = (PSV−EDV)/PSV) of the measured vessels were computed for every patient. The ophthalmic artery was traced about 10–15 mm behind the globe, just nasal to the optic nerve after their crossing. The CRA was depicted in the anterior part of the optic nerve shadow, about 2–3 mm behind the surface of the optic disc.

Statistical analysis

Results are presented as mean (standard deviation (SD)). The Kolmogorov–Smirnov test was used to determine the normal distribution of the variables. The paired-samples t test was used for comparing the measurements before and after treatment with atorvastatin or placebo in each group. Bivariate correlation between the blood flow velocities and the lipid measures was performed using Pearson's correlation coefficient. For comparison of the groups for baseline and after 10 weeks measurements, analysis of variance and Tukey's retrospective analyses was used.

Table 1 Baseline characteristics of the patients

	Atorvastatin		Placebo group 3 (n = 15)	
	Group 1 NPDR (n = 15)	Group 2 PDR (n = 15)	NPDR (n = 8)	PDR (n = 7)
Age (years)	56.4 (7.1)	60.8 (7.7)	57.5 (8.0)	58.6 (8.7)
Sex (M/F; n)	8/7	10/5	3/5	4/3
BMI (kg/m^2)	26.9 (3.1)	27.5 (3.4)	27.2 (4.0)	27.4 (3.9)
Heart rate (beats/min)	73.7 (8.0)	75.0 (8.1)	74.1 (8.6)	74.8 (8.9)
Weight (kg)	69.1 (11.4)	76.0 (14.3)	75.0 (15.4)	75.3 (15.0)
Duration of diabetes (years)	9.4 (3.1)	16.7 (3.4)	12.9 (3.8)	15.0 (4.3)
HbA_{1C} (%)	7.6 (1.5)	8.2 (1.6)	7.7 (1.7)	8.0 (1.7)
Fasting plasma glucose (mg/dl)	145.2 (18.4)	160.5 (20.6)	158.4 (25.3)	159.7 (24.4)
Blood pressure(mm Hg)				
Systolic	115.4 (10.7)	121.1 (11.2)	117.8 (9.8)	119.2 (10.2)
Diastolic	78.2 (7.6)	80.4 (8.1)	79.4 (7.4)	81.6 (8.5)
Ocular perfusion pressure (mm Hg)	46.6 (3.1)	48.7 (4.0)	47.2 (3.4)	48.0 (3.7)

BMI, body mass index; F, female; HbA_{1C}, glycated haemoglobin; M, male; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.
Values are mean (SD) unless otherwise specified.

Table 2 Changes in lipid levels in patients with type 2 diabetes mellitus after 10 weeks of treatment with atorvastatin or placebo

	Group 1 (n = 15)			Group 2 (n = 15)			Group 3 (n = 15)					
							NPDR (n = 8)			PDR (n = 7)		
	Baseline	After 10 weeks	p Value*	Baseline	After 10 weeks	p Value*	Baseline	After 10 weeks	p Value*	Baseline	After 10 weeks	p Value*
TC	180.2 (10.1)	153.2 (7.3)	<0.001	191.6 (14.3)	150.6 (10.1)	<0.001	178.1 (14.2)	173.8 (11.7)	0.340	178.2 (11.6)	174.2 (14.0)	0.546
LDL-C	133.4 (8.6)	98.9 (8.6)	<0.001	133.0 (10.9)	102.1 (9.3)	<0.001	127.5 (9.9)	124.2 (10.1)	0.443	126.2 (7.0)	125.4 (6.7)	0.824
HDL-C	41.9 (8.7)	40.7 (8.40	0.645	40.0 (8.6)	39.8 (8.3)	0.720	40.8 (8.6)	38.8 (9.7)	0.237	37.2 (4.7)	39.0 (8.8)	0.345
TG	140.6 (7.3)	111.9 (11.4)	<0.001	148.0 (12.1)	115.4 (12.0)	<0.001	151.2 (14.8)	146.7 (14.1)	0.211	153.1 (15.7)	147.8 (13.5)	0.119

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; TC, total cholesterol; TG, triglycerides.
Values are mean (SD).
*p Value for comparison before and after atorvastatin and placebo treatment.

RESULTS

Baseline subject characteristics

Table 1 gives the pretreatment characteristics of the patients in each group. Atorvastatin was generally well tolerated, with no effect on the measures of diabetic control or any adverse events.

Effect of atorvastatin on lipid levels

Table 2 shows lipid levels at baseline and after atorvastatin and placebo treatment. Atorvastatin significantly decreased total cholesterol, low-density lipoprotein cholesterol and triglyceride levels in groups 1 and 2 ($p<0.001$ for both), but no significant changes were observed in high-density lipoprotein cholesterol levels ($p>0.05$ for both). We found no significant differences in lipid levels after placebo treatment ($p>0.05$ for both).

Effect of atorvastatin on haemodynamic parameters

Table 3 shows the measurements for ocular blood flow before and after treatement in each group. The baseline haemodynamic parameters were similar between the atorvastatin and placebo groups ($p>0.05$ for both). After atorvastatin treatment, the mean PSV of the ophthalmic artery in group 2 ($p = 0.007$) and the mean PSV of the CRA in groups 1 ($p = 0.002$) and 2 ($p = 0.01$) decreased significantly, but the mean EDV of the ophthalmic artery and CRA did not change in CRA, central retinal artery; group ($p>0.05$ for both), and the mean resistive index of the CRA was significantly lower after atorvastatin treatment in groups 1 ($p = 0.003$) and 2 ($p = 0.01$). Although there was a slight decrease in the mean resistive index of the ophthalmic artery after atorvastatin treatment in group 2, the difference was not significant ($p = 0.06$). We found no significant differences in haemodynamic parameters after placebo treatment in group 3 compared with pretreatment values ($p>0.05$ for both). After 10 weeks of atorvastatin treatment, the PSV of the ophthalmic artery ($p = 0.01$) and CRA ($p = 0.03$), and the resistive index of the CRA ($p = 0.03$) in group 2 were significantly lower compared with the corresponding values in patients with PDR who received placebo. In addition, significant correlations were observed between baseline total cholesterol and the EDV of the ophthalmic artery ($r = -0.522$; $p = 0.046$), and total cholesterol after 10 weeks of atorvastatin treatment and the resistive index of the ophthalmic artery ($r = 0.682$; $p = 0.005$) in group 1.

DISCUSSION

Although the effect of diabetes on ocular circulation is not fully understood, altered retinal circulation is well documented. In the eyes of people with diabetes, retinal vessels do not show the same autoregulatory response as in the eyes of healthy people. Several studies have reported both increased[25] and decreased[26] retinal blood flow in patients with diabetes mellitus compared with healthy controls. This discrepancy is of particular importance because the judgement as to whether the drug might harm or benefit ocular circulation remains completely speculative.

Findl *et al*[27] did not observe any difference between patients with diabetes and controls using laser Doppler velocimetry. Using the same method, however, Grunwald *et al*[28] showed that retinal blood flow increased in patients with diabetes without retinopathy compared with that in controls. Goebel *et al*[29] found considerably lower PSV and EDV values in the CRA of patients

Table 3 Comparison of mean blood flow velocities (cm/s) in patients with diabetes before and after atorvastatin and placebo treatment

	Group 1 (n = 15)			Group 2 (n = 15)			Group 3 (n = 15)					
							NPDR (n = 8)			PDR (n = 7)		
	Baseline	After 10 weeks	p Value*	Baseline	After 10 weeks	p Value*	Baseline	After 10 weeks	p Value*	Baseline	After 10 weeks	p Value*
OA												
PSV	32.5 (5.8)	29.5 (5.0)	0.053	30.0 (7.1)	25.2 (5.6)	0.007†	33.6 (6.5)	32.6 (6.7)	0.57	30.5 (5.7)	31.1 (5.5)	0.81
EDV	6.7 (1.4)	6.5 (1.1)	0.32	6.3 (1.3)	6.1 (1.4)	0.43	6.7 (1.2)	6.6 (1.1)	0.52	6.3 (1.6)	6.3 (1.4)	0.91
RI	0.80 (0.06)	0.77 (0.05)	0.12	0.79 (0.05)	0.77 (0.04)	0.06	0.78 (0.06)	0.79 (0.06)	0.62	0.80 (0.06)	0.79 (0.05)	0.67
CRA												
PSV	11.3 (1.9)	9.5 (1.4)	0.002†	9.2 (2.0)	7.8 (1.6)	0.01†	10.5 (2.1)	10.4 (2.0)	0.90	9.2 (1.9)	9.3 (2.0)	0.67
EDV	3.4 (0.7)	3.5 (0.5)	0.78	3.0 (0.6)	3.0 (0.4)	0.75	3.3 (0.4)	3.4 (0.5)	0.66	3.1 (0.5)	3.0 (0.4)	0.76
RI	0.69 (0.06)	0.62 (0.06)	0.003†	0.66 (0.08)	0.61 (0.08)	0.01†	0.68 (0.07)	0.69 (0.07)	0.88	0.66 (0.08)	0.67 (0.07)	0.90

CRA, central retinal artery; EDV, end diastolic velocity; NPDR, non-proliferative diabetic retinopathy; OA, Opthalmic artery; PDR, proliferative diabetic retinopathy; PSV, Peak systolic velocity; RI, resistive index.
Values are mean (SD).
* p Value for comparison before and after atorvastatin and placebo treatment.
† Statistically significant.

with untreated diabetic retinopathy than in controls. Similar results were reported later by MacKinnon *et al*.[30] Dimitrova *et al*[31] reported a marked increase in central retinal vein velocity and resistive index in patients with diabetic retinopathy compared with controls and in patients with diabetes without diabetic retinopathy. The CRA and posterior ciliary artery did not show marked differences between the initial and final measurements. Considerably increased retrobulbar central retinal vein velocity was also reported by Mendivil *et al*[4] in patients with PDR, and by Li Ping[32] in patients with background diabetic retinopathy and PDR. However, Guven *et al*[33] did not find similar results using CDI.

We showed that there was no significant difference in ocular blood flow between patients with NPDR and in those with PDR ($p<0.05$ for both). After atorvastatin treatment, we observed that the mean PSV of the ophthalmic artery in patients with PDR decreased considerably. The mean PSV of the CRA also decreased without any changes in the mean EDV of patients with NPDR or in those with PDR who had normal cholesterol levels. After 10 weeks of treatment with atorvastatin, the resistive index of the CRA also decreased markedly in patients with diabetes.

Lipids participate in the pathophysiology of diabetic vascular disease, and lipid abnormalities are common in patients with type 2 diabetes. Endothelial dysfunction is one of the characteristic features of diabetes mellitis in both animal models and humans, and its reversal is reported to retard the development of vascular disease.[13 14] An important cause of endothelial dysfunction is the reduced bioavailability of endothelium-dependent nitric oxide. Indeed, oxidised low density lipoprotein reduces nitric oxide synthase expression, enhances nitric oxide breakdown and impairs endothelial-dependent dilatation.[34] Atorvastatin was shown to be effective in preventing the oxidisation of low density lipoprotein in patients with normal cholesterol levels, whereas statins directly decrease the expression of endothelin 1, increase the activity of endothelial nitric oxide synthase and, therefore, improve endothelial function.[35] Previous studies also showed that treatment with atorvastatin improved endothelial dysfunction and also increased flow-mediated endothelium-dependent dilatation as a response to reactive hyperaemia in patients with primary hypercholesterolaemia.[36] Simvastatin is one of the family of statins, HMG-CoA reductase inhibitors, which can suppress blood–retinal barrier breakdown in the retina of the patients with diabetes, by inhibiting leucocyte–endothelial cell interactions through suppressing the expression of intercellular adhesion molecule 1 on endothelial cells, and thereby prevent any subsequent retinal vascular damage and blood–retinal barrier breakdown in the retina of people with diabetes.[37] Gordon *et al*[38] and Sen *et al*[39] reported that simvastatin retards the progression of diabetic retinopathy. Miyahara *et al*[37] also reported that simvastatin reduces expression of vascular endothelial growth factor (VEGF), which plays a crucial part in diabetic retinopathy. Weis *et al*[40] reported that endothelial release of the VEGF was considerably decreased with high concentrations of statins.

In this study, we selected people with normal cholesterol levels to examine the effect of atorvastatin on ocular blood flow as a potential complementary approach. Such a rapid response in ocular blood flow velocities seems to correlate with the improvement in vascular resistance and possibly endothelial function, which was reported in patients with hypercholesterolaemia after 2 weeks of treatment with atorvastatin.[16] Our study also provides the first evidence that atorvastatin might decrease the mean PSV of the CRA in patients with diabetes without changing the mean EDV as it improves the vascular resistance. Although the resistive index changed in the CRA, the same change was not observed in the ophthalmic artery. This may be related with the small number of the sample size ($p = 0.06$). An important aspect of this finding is that retinal hypoxia and the susceptibility of capillary walls weakened by loss of pericytes to further damage might decrease because of the improvement in vascular resistance. The decrease in pressure to the capillary wall may prevent the formation of microaneurysms, microvascular leakage or haemorrhage from new vessels. As a result, atorvastatin may play an important part in maintaining the integrity of the retinal vasculature and, therefore, reduce the retinal complications of type 2 diabetes mellitis.

Our study has two limitations: (1) The sample size is relatively small and (2) the patients were not randomly allocated to treatment and control arms.

CONCLUSION

Atorvastatin decreases the mean PSV of the ophthalmic artery in patients with PDR, and the mean PSV and resistive index of the CRA in patients with NPDR or in those with PDR, without any changes in the mean EDV of the ophthalmic artery and the CRA. The benefit or harm of a drug on ocular circulation cannot be predicted easily; further examinations with large numbers of patients are therefore required to obtain the long-term results of this drug.

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