

## Clinical Study

# The Effect of Amlodipine on Oxidative Stress in Patients with Type 2 Diabetes

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*Background.* Amlodipine, a calcium channel blocker, is reported to have an antioxidative effect in vitro, but whether such an effect occurs clinically is unknown. The purpose of the study was to investigate the effect of amlodipine (5 mg/day) on oxidative stress by measuring urinary 8-iso-prostaglandin F<sub>2</sub>α (8-iso-PGF<sub>2</sub>α), an oxidative stress marker, in patients with type 2 diabetes. The anti-oxidative effect of amlodipine was also assessed using the levels of high-sensitivity C-reactive protein (hsCRP) and fibrinogen. *Patients and Methods.* Seventeen consecutive patients with type 2 diabetes complicated by hypertension were prospectively enrolled in the study. These patients received amlodipine (5 mg/day) over a 3-month period and various markers were measured before and at the end of this period. *Results.* Urinary 8-iso-PGF<sub>2</sub>α showed a tendency to decrease, but the change was not statistically significant ( $P = .1127$ ). The patients were divided into two groups according to the baseline level of urinary 8-iso-PGF<sub>2</sub>α, and a significant decrease in 8-iso-PGF<sub>2</sub>α was found in the group ( $n = 9$ ) with a relatively high baseline 8-iso-PGF<sub>2</sub>α. There were no changes in hsCRP, fibrinogen, and plasminogen activator inhibitor-1. Significant decreases in systolic and diastolic blood pressure were observed ( $P < .0001$ ,  $P = .0151$ , resp.). *Conclusion.* The results show that amlodipine (5 mg/day) may provide a clinically useful anti-oxidative effect, based on evaluation of urinary 8-iso-PGF<sub>2</sub>α.

## 1. Introduction

Oxidative stress induces endothelial dysfunction and subsequent progression of atherosclerosis [1]. Antihypertensive drugs such as angiotensin II receptor blockers (ARBs) have been shown to provide an antioxidative effect [2], and such drugs might therefore have protective effects for organs independently of antihypertensive activity. Similarly, amlodipine, a representative antihypertensive drug from the family of calcium channel blockers, has specific antioxidative and antiinflammatory effects in vitro [3, 4]. However, the clinical relevance of these effects has not been determined.

We have previously investigated the effect of amlodipine (2.5 mg/day) on urinary 8-iso-prostaglandin F<sub>2</sub>α (8-iso-PGF<sub>2</sub>α), a marker of systemic oxidative stress [5, 6], in patients with hypertension and type 2 diabetes complicated by diabetic nephropathy, but a significant decrease in 8-iso-PGF<sub>2</sub>α was not observed [7]. We speculate that the dose of amlodipine was too small to give a clinically apparent antioxidative effect. Therefore, in the current study, we examined the effect of amlodipine at a dose of 5.0 mg/day on urinary

8-iso-PGF<sub>2</sub>α, on low-grade inflammation evaluated by high-sensitivity-C-reactive protein (hsCRP) and fibrinogen, and on plasminogen activator inhibitor (PAI) 1, a major inhibitor of fibrinolysis. In performing the study, we hypothesized that administration of amlodipine (5.0 mg/day) would result in antioxidative and antiinflammatory effects and would also decrease PAI-1.

## 2. Patients and Methods

*2.1. Patients.* Initially, 20 outpatients with type 2 diabetes complicated by hypertension (systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg) were enrolled in the study. Of these patients, 3 were subsequently excluded from the study: 1 showed apparent symptoms of a common cold on their second visit and 2 had poor compliance at followup. Therefore, the final study included 17 patients who received amlodipine. A control group using a placebo was not used, since no effect of amlodipine (2.5 mg/day) on urinary 8-iso-PGF<sub>2</sub>α was found in our previous study [7]. Twenty-five age-matched

TABLE 1: Clinical features in nondiabetic control and amlodipine groups.

	Nondiabetes	Amlodipine	P value
No. (men/women)	25 (9/16)	17 (8/9)	—
No. (ARB/ACE-I)	—	6 (4/2)	—
Age (year)	62.8 ± 4.9	64.6 ± 11.6	.1412
Duration (year)	—	12.3 ± 7.4	—
FPG (mg/dL)	98.0 ± 9.7	146.6 ± 72.0	.0138*
HbA <sub>1C</sub> (%)	4.9 ± 0.3	7.3 ± 1.1	<.0001*
SBP (mm Hg)	124.0 ± 8.9	150.7 ± 8.9	<.0001*
DBP (mm Hg)	73.8 ± 6.7	85.1 ± 19.2	.0325*

All data are expressed as mean ± standard deviation (SD). The two time points for an individual were compared by the use of a paired *t*-test.

*P*: *P* value; *P* < .05 are defined as statistical significance (\*).

ARB: angiotensin II receptor blocker; ACE-I: angiotensin converting enzyme inhibitor; FPG: fasting plasma glucose; HbA<sub>1C</sub>: hemoglobin; SBP: systolic blood pressure; DBP: diastolic blood pressure.

healthy subjects were enrolled as controls for baseline measurements. Concomitant antihypertensive drugs (except for other calcium channel blockers) were permitted, but all drugs regimens were unchanged from 1 month before the study until the end of the 3-month period of the study. Total six patients had concomitant antihypertensive drugs (four patients had ARB, and 2 patients had angiotensin-converting enzyme inhibitor (ACE-I)). Patients exhibiting evidence of liver dysfunction or findings of infectious or autoimmune disease were excluded from the study. The clinical features of the nondiabetic controls and the patients are shown in Table 1.

**2.2. Methods.** Consecutive patients were prospectively enrolled as they visited our outpatient department. These patients received amlodipine over a 3-month period. Blood and urine tests were performed at the beginning of this period (0 weeks) and after 12 weeks (3 months). For patients and healthy subjects, blood and urine were sampled in the outpatient department from 8:30 to 9:30 A.M. after overnight fasting for at least 10 hours. The collected blood was immediately divided into test tubes for the respective measurements (see below) and then centrifuged at 1,500 rpm for 5 minutes to separate serum or plasma from clot-containing blood cells. Except for samples for glucose and HbA<sub>1C</sub> measurements, the blood and urine samples were stored frozen at -70°C until analysis. At the time of sampling, the patients were weighed in their underwear.

**Urinary 8-Iso-PGF2 $\alpha$  Assay.** Urinary 8-iso-PGF2 $\alpha$  was measured in morning urine with an enzyme immunoassay (EIA) kit (ACE EIA; Cayman Chemical Company, Ann Arbor, MI). The intra- and interassay CVs were less than 10%, based on actual values. This assay has been reported to show no differences in measurements of morning urine samples analyzed immediately or after storage for 24 hours [8]. To diminish the potential effects of exercise on diabetes on the day before measurement of these markers, we used real values (rather than corrected for creatinine).

**Plasma PAI-1.** The plasma concentration of PAI-1 was measured using plasma (stored frozen) in a test tube containing

sodium citrate, using an ELISA (Biopool Immulyse PAI-1; Biopool, Umea, Sweden) that detects both active and latent PAI-1, as well as PAI-1 bound to t-PA. The intra- and inter-assay CVs were 2.26% to 3.77% and 3.57% to 4.76%, respectively.

**Other Measurements.** Plasma glucose, HbA<sub>1C</sub>, serum lipid concentrations, and serum hsCRP were measured as described previously [9].

**Ethical Considerations.** All subjects gave informed consent to inclusion in the study, which was performed according to the guidelines proposed in the Declaration of Helsinki.

**Statistical Methods.** All data are presented as means ± standard deviation (SD), except for the 8-iso-PGF2 $\alpha$ , hsCRP and urinary albumin excretion (UAE), all of which showed a skewed distribution and are presented as geometric means with interquartile ranges (25th and 75th). Comparison of two time points for an individual was performed using a paired *t*-test or Wilcoxon signed rank test (for 8-iso-PGF2 $\alpha$ , hsCRP, and UAE). Comparison of 8-iso-PGF2 $\alpha$  levels between two groups was performed using a Wilcoxon rank sum test. A *P* value of less than .05 was considered to indicate statistical significance.

### 3. Results

There was a tendency for a significant elevation of baseline urinary 8-iso-PGF2 $\alpha$  levels in the 17 patients with type 2 diabetes, compared with the 25 control subjects (*P* = .1243). After three months of amlodipine therapy, the 8-iso-PGF2 $\alpha$  level showed a tendency for a decrease in the 17 patients, but the difference was not statistically significant: 126.7 (69.5, 215) to 96.2 (52.5, 160) ng/mL, *P* = .1127. However, when the patients were divided into two groups based on the median level of urinary 8-iso-PGF2 $\alpha$  at baseline, the group (*n* = 9) with a relatively high baseline 8-iso-PGF2 $\alpha$  level did show a significant decrease in 8-iso-PGF2 $\alpha$  after 3 months: 210.1 (130, 350) to 116.9 (61.5, 200) ng/mL, *P* = .0208. There was also a significant decrease in systolic

TABLE 2: The changes in variables by 3-months amlodipine therapy in 17 diabetic patients.

	Base line	3 months	P value
8-iso-PGF2 $\alpha$ (pg/mL)	126.7 (69.5, 215)	96.2 (52.5, 160)	.1127
8-iso-PGF2 $\alpha$ (pg/mL)	210.1 (130, 350)	116.9 (61.5, 200)	.0208*
(Subgroup composed of patents with high values of 8-iso-PGF2 $\alpha$ : n = 9)			
HsCRP (mg/L)	0.9095 (0.3005, 3.810)	0.9974 (0.405, 2.800)	.6191
PAI-1 (ng/mL)	27.7 $\pm$ 3.8	24.9 $\pm$ 19.1	.6991
Fibrinogen (mg/dL)	365.5 $\pm$ 62.0	380.6 $\pm$ 70.4	.1441
FPG (mg/dL)	146.6 $\pm$ 72.0	159.3 $\pm$ 55.6	.5193
HbA <sub>1C</sub> (%)	7.3 $\pm$ 1.1	7.6 $\pm$ 1.3	.0760
SBP (mm Hg)	150.7 $\pm$ 8.9	127.9 $\pm$ 15.6	<.0001*
DBP (mm Hg)	85.1 $\pm$ 19.2	72.6 $\pm$ 6.4	.0151*
UAE (mg/g.Cr)(n=14)	201.6 (80.5, 469)	281.9 (90.5, 941.5)	.5936

All data are expressed as mean  $\pm$  standard deviation (SD). The two time points for an individual were compared by use of a paired *t*-test. *P*: *P* value; *P* < .05 are defined as statistical significance (\*). 8-iso-PGF2 $\alpha$ : 8-iso-prostaglandin F2 $\alpha$ ; hsCRP: high-sensitivity C reactive protein; PAI-1: plasminogen activator inhibitor; FPG: fasting plasma glucose; HbA<sub>1C</sub>: hemoglobin A<sub>1C</sub>; SBP: systolic blood pressure; DBP: diastolic blood pressure; UAE: urinary albumin excretion.

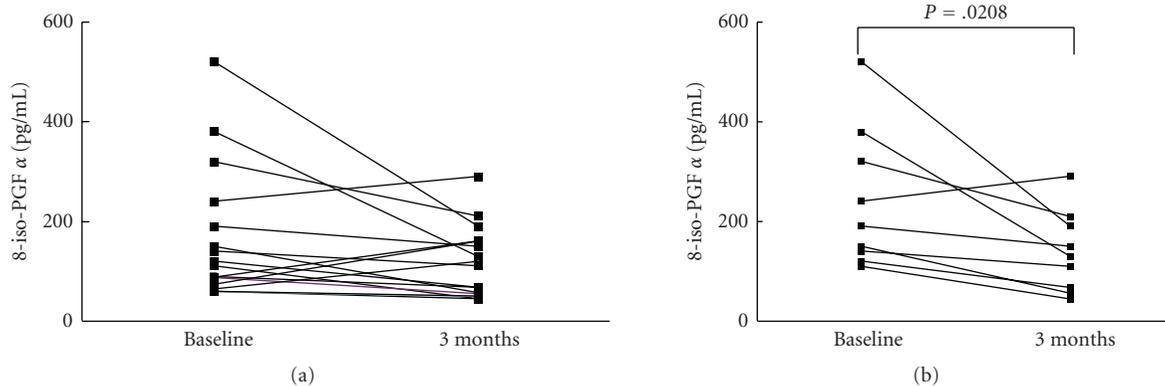


FIGURE 1: The effect of amlodipine on 8-iso-PGF2- $\alpha$  in the all patients with type 2 diabetes (a) (n = 17), (b), who had a relatively high baseline 8-iso-PGF2- $\alpha$  (n = 9).

and diastolic pressure in all 17 patients after 3 months of amlodipine therapy, but no changes in hsCRP, fibrinogen, or PAI-1. The changes in the variables following amlodipine therapy are shown in Table 2, and the changes in the 8-iso-PGF2 $\alpha$  level caused by amlodipine administration in all 17 patients and in the 9 patients with relatively high levels of 8-iso-PGF2 $\alpha$  at baseline are shown in Figures 1(a) and 1(b), respectively. There was no significant correlation between differences in the baseline and 3-month levels of 8-iso-PGF2 $\alpha$  and differences in baseline and 3-month SBP or DBP in the 17 patients, or in the 8 patients with low 8-iso-PGF2 $\alpha$  and the 9 patients with high 8-iso-PGF2 $\alpha$  (data not shown). Furthermore, to investigate the factors associated with the increase in oxidative stress in the 9 patients in whom amlodipine reduced oxidative stress, we assessed the correlation between 8-iso-PGF2 $\alpha$  and age, diabetic duration, body weight, FPG, HbA<sub>1C</sub>, SBP, DBP, hsCRP, PAI-1, Fibrinogen, or serum lipid concentrations (low- and high-density lipoprotein and triglyceride) in baseline in these 9 patients; however there were no significant correlation between 8-iso-PGF2 $\alpha$  and these markers (data not shown). In 2 patients treated with amlodipine in addition to ACE-I (these patients were included in patients with high 8-iso-

PGF2 $\alpha$  group), by 3-month therapy with these drugs, 8-iso-PGF2 $\alpha$  reduced from 520, 190 to 110, 44 pg/mL, respectively (63% reduction in mean), while in 4 patients (only one patients belonged to patients-group with high 8-iso-PGF2 $\alpha$ ) who received both ARB and amlodipine, only 5% reduction in mean was found. The other 11 patients who received amlodipine alone showed 20% reduction in mean for 8-iso-PGF2 $\alpha$  by 3 months-therapy.

#### 4. Discussion

Amlodipine has an antioxidative effect on cell membranes in vitro [3], but the clinical effect of amlodipine on oxidative stress is unclear. In the current study, we found a tendency for a decrease in urinary 8-iso-PGF2 $\alpha$ , a systemic oxidative stress marker [5, 6], in patients with type 2 diabetes following amlodipine administration at 5 mg/day for 3 months, although the difference did not achieve statistical significance. This finding appears to be in contrast with our previous study [7], in which no change of 8-iso-PGF2 $\alpha$  was observed following therapy with amlodipine at a dose of 2.5 mg/day. Therefore, we speculate that the

clinical antioxidative effect of amlodipine might be dose-dependent and that a dose of amlodipine of at least 5 mg/day might be needed to show a full clinical antioxidative effect. Interestingly, when the patients were divided into two groups based on the level of urinary 8-iso-PGF2 $\alpha$  at baseline, a significant decrease in urinary 8-iso-PGF2 $\alpha$  due to amlodipine therapy was obtained in patients with a relatively high baseline level of urinary 8-iso-PGF2 $\alpha$ .

Urinary 8-iso-PGF2 $\alpha$  is reported to be elevated in diabetic patients [10] and our data also showed a tendency for significant elevation of urinary 8-iso-PGF2 $\alpha$  in diabetic patients, compared with healthy controls. Therefore, our finding that amlodipine lowered urinary 8-iso-PGF2 $\alpha$  more significantly in patients with a relatively high baseline level of urinary 8-iso-PGF2 $\alpha$  may suggest that amlodipine therapy is more effective for inhibition of oxidative stress in subjects in whom elevated oxidative stress is likely, such as diabetic patients [10, 11]; however we could not detect concrete factors associated with increase in oxidative stress in patients with relatively high baseline level of urinary 8-iso-PGF2 $\alpha$  because 8-iso-PGF2 $\alpha$  in baseline in these patients did not correlate with various factors, including FPG and HbA<sub>1C</sub>, which may potentially increase oxidative stress. Since oxidative stress is associated with the progression of atherosclerosis [1], this also suggests that amlodipine might be beneficial in inhibition of progression of atherosclerosis. However, since amlodipine simultaneously decreased blood pressure, the antioxidative effect of amlodipine may be associated with an effect on blood pressure; in fact, in the current study, the decrease in systolic blood pressure was large, compared with that in our previous study using a lower dose of amlodipine [7]. However, there was no correlation between the difference in urinary 8-iso-PGF2 $\alpha$  and that in SBP or in DBP before and after amlodipine therapy in the current study, suggesting that the influence of blood pressure on oxidative stress was not strong.

In the current study, inflammatory markers such as hsCRP or fibrinogen were unaffected by amlodipine therapy. It is reported that amlodipine in vitro has a local anti-inflammatory effect via inhibition of adhesion of monocytes to endothelial cells [4], but our results suggest that amlodipine does not have clinically apparent systemic anti-inflammatory effects, based on hsCRP and fibrinogen levels. In addition to the lack of an effect on fibrinogen, which is also a major coagulant factor, amlodipine had no effect on PAI-1, a major inhibitor of fibrinolysis, suggesting little effect of amlodipine on the coagulant system.

In the current study, patients with concomitant use of amlodipine and ACE-I had larger reduction of 8-iso-PGF2 $\alpha$  compared with those with concomitant use of amlodipine and ARB or amlodipine alone, although the patients with concomitant use of ACE-I were only 2 patients. Interestingly, there are reports that the combination of amlodipine and ACE-I increases nitric oxide availability more than either alone [12, 13]; this may be at least partially explain the above result in the current study. Furthermore, a recent study showed that amlodipine and ACE-I (benazapril) were superior to the benazapril and hydrochlorothiazide (diuretic) in reducing cardiovascular events in patients with

hypertension who were at high risk for such events [14]. Therefore, it would be interesting to investigate whether combination therapy by amlodipine and ACE-I can achieve larger reduction of oxidative stress in future studies with large sample size. Finally, in view of the fact that there appears to be a dose-related effect of amlodipine, it might have been of interest to see if larger dose of amlodipine such as 10 mg might have reduced oxidative stress in those patients in the present study in which it appeared ineffective.

In conclusion, amlodipine therapy at 5.0 mg/day showed a tendency to decrease urinary 8-iso-PGF2 $\alpha$  in patients with type 2 diabetes and caused a significant decrease in urinary 8-iso-PGF2 $\alpha$  in patients with a high baseline level of 8-iso-PGF2 $\alpha$ . Amlodipine therapy had no effects on inflammatory markers such as hsCRP and fibrinogen. Our findings suggest that amlodipine at 5.0 mg/day may provide a clinically relevant antioxidative effect, but it is unclear if this dose of amlodipine exerts a clinical benefit independently of the concomitant decrease in blood pressure.

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