Metformin Treatment Lowers Asymmetric Dimethylarginine Concentrations in Patients With Type 2 Diabetes

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This study was initiated to see if plasma asymmetric dimethylarginine (ADMA) concentrations decreased in hyperglycemic patients with type 2 diabetes following metformin treatment, either as monotherapy or following its addition to sulfonylurea-treated patients. Fasting plasma glucose, dimethylarginine, and L-arginine concentrations were measured before and 3 months after the administration of a maximally effective dose of metformin to 31 patients with type 2 diabetes in poor glycemic control (fasting plasma concentrations > 9.7 mmol/L), while being treated with either diet (n = 16) or a maximal amount of a sulfonylurea compound (n = 15). Fasting plasma glucose concentration (mean ± SEM) decreased to a similar degree ($P < .01$) in patients treated with either metformin alone (12.4 ± 0.5 to 9.5 ± 0.5 mmol/L) or when it was added to a sulfonylurea compound (14.1 ± 0.5 to 10.6 ± 0.9 mmol/L). The improvement in glycemic control was associated with similar decreases ($P < .01$) in ADMA concentrations in metformin (1.65 ± 0.21 to 1.18 ± 0.13 μmol/L) and sulfonylurea + metformin-treated patients (1.75 ± 0.13 to 1.19 ± 0.08 μmol/L). Plasma L-arginine concentrations were similar in the 2 groups at baseline and did not change in response to metformin. Thus, metformin treatment was associated with a favorable increase in the plasma L-arginine/ADMA ratio. These results provide the first evidence that plasma ADMA concentrations decrease in association with improved glycemic control in patients with type 2 diabetes and demonstrate that the magnitude of the change in metformin-treated patients was similar, irrespective of whether it was used as monotherapy or in combination with sulfonylurea treatment.

THE RESULTS OF THE United Kingdom Prospective Diabetes Study (UKPDS) of patients with type 2 diabetes demonstrated that microangiopathy improves to a greater degree with better glycemic control than does macrovascular disease.[1] The reason for the disparity between the effects of glycemic control on microvascular versus macrovascular diseases is not apparent, but it is now clear that patients with type 2 diabetes have multiple coronary heart disease (CHD) risk factors.[2] The present study was initiated to evaluate the potential importance of a less well-recognized CHD risk factor, asymmetric dimethylarginine (ADMA). ADMA is an endogenous competitive inhibitor of nitric oxide synthase (NOS)[3] and has been shown to be increased in animals and patients with hypercholesterolemia and atherosclerosis.[4-6] Importantly, the elevations in plasma ADMA, and the resultant disruptions in L-arginine/ADMA ratios, are associated with a clinically relevant decrement in endothelial function. Indeed, plasma ADMA concentrations correlate better with endothelium-dependent vasodilation than do low-density lipoprotein (LDL) cholesterol concentrations.[4] Furthermore, recent evidence has shown that ADMA concentrations were directly correlated with plasma glucose concentrations in a population that included diabetics and nondiabetics,[7] and we have presented evidence that plasma ADMA concentrations are elevated in patients with type 2 diabetes.[8]

Given this background, we initiated the current study to see if plasma ADMA concentration would change with improved glycemic control. The decision to assess the effect of improved glycemic control with metformin on plasma ADMA concentration was based on the finding in the UKPDS substudy analysis of overweight patients that patients assigned to intensive treatment with metformin had a significant decrease in myocardial infarction, as well as in diabetes-related death, whereas the addition of metformin to patients already treated with a sulfonylurea compound did not lead to a decrease in myocardial infarction rate, and diabetes-related death was actually increased in these patients.[1] Consequently, we have quantified the effects of metformin treatment on glycemic control and plasma ADMA, given either as monotherapy or added to a sulfonylurea compound.

MATERIALS AND METHODS

The study was approved by the Stanford Human Subjects Committee, and each volunteer gave written informed consent before entering the General Clinical Research Center (GCRC). The study population consisted of 31 patients with type 2 diabetes: 15 who were in relatively poor glycemic control on maximal sulfonylurea treatment and 16 patients with type 2 diabetes with uncontrolled hyperglycemia in the absence of specific antihyperglycemic drug treatment. The 2 groups were similar in terms of age (mean ± SD; 56 ± 11 *v* 58 ± 9 years), known duration of diabetes (5 ± 2 *v* 5 ± 6 years), and gender distribution (M/F = 11/4 *v* 11/5). There was no evidence of diabetic vascular complications in any patient, with the exception of a few cases of background retinopathy. Furthermore, blood count and chemical screening battery were normal in all subjects. Fasting plasma ADMA concentrations were similar in both groups and higher than values we have previously reported for nondiabetic controls.[8]

Subjects satisfying the initial screening criteria were seen by a physician member of the research group at weekly intervals for at least 4 weeks and maintained on their initial program of diet or diet plus sulfonylurea treatment. At the end of this period, they were admitted to the GCRC for baseline measurements before the initiation of treatment with metformin.

After an overnight fast, blood was drawn for measurement of plasma glucose as previously described.[9] Plasma concentrations of ADMA

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Table 1. Effect of Metformin Treatment on Anthropometric and Metabolic Variables

Variable	Metformin			SU + Metformin		
	Before	After	*P*	Before	After	*P*
Weight (kg)	87.6 ± 4.2	86.7 ± 4.2	NS	91.1 ± 6.8	92.1 ± 6.9	NS
Systolic BP (mm Hg)	147 ± 4	139 ± 4	NS	136 ± 4	138 ± 5	NS
Diastolic BP (mm Hg)	81 ± 2	79 ± 2	NS	81 ± 3	80 ± 2	NS
Glucose (mmol/L)	12.4 ± 0.5	9.5 ± 0.5	<.001	14.1 ± 0.5	10.6 ± 0.9	<.001
HbA_{1c} (%)	10.6 ± 0.4	9.6 ± 0.5	<.05	13.1 ± 0.8	10.7 ± 0.7	<.01
Insulin (pmol/L)	111 ± 23	128 ± 32	NS	128 ± 27	110 ± 19	NS

Abbreviations: SU, sulfonylurea; BP, blood pressure; HbA_{1c}, glycosylated hemoglobin.

were measured by high-performance liquid chromatography (HPLC) with precolumn derivatization with o-phthaldialdehyde (OPA) using a modification of a previously described method.[5] Briefly, 0.5 mL of sample was spiked with 1×10^{-5} mol/L L-homoarginine as an internal standard and ADMA isolated by solid-phase extraction with a cation-exchange column (Bond Elute SCX50mg; Varian Associates, Palo Alto, CA) after protein precipitation according to Pettersson et al.[10] The eluates were evaporated to dryness at 50°C under nitrogen and resuspended in double-distilled water. HPLC was performed on a computer-controlled chromatography system (Varian Star) consisting of an HPLC pump (Varian 9010), an automatic injector with sample-reagent mixing capabilities (Varian 9100) and a fluorescence detector (Varian Fluorichrome II). The samples were incubated for exactly 1 minute with OPA reagent (5.4 mg/mL OPA in borate buffer pH = 8.4, containing 0.4% b-mercaptoethanol) before automatic injection into the HPLC. The OPA derivatives of L-arginine, ADMA, symmetric dimethylarginine (SDMA), and the internal standard L-homoarginine were separated on a 250 × 4.5 mm-ID 7 μm Nucleosil Phenyl HPLC column (Supelco, Bellafonte, PA) with the flurescence detector set at 340 nm excitation and 450 nm emission. Amino acids were eluted from the column with an isocratic gradient of 50 mmol/L K-phosphate buffer (pH = 6.6)/90% methanol (80:20) at a flow rate of 1 mL/min. ADMA and SDMA concentrations were calculated by comparing the ADMA/homoarginine ratio with standards of known concentrations. The recovery rate for ADMA was 85% and the intrasample variation was 4%. The detection limit of the assay was 0.1 μmol/L.

Following these baseline determinations, patients were started on 500 mg of metformin (N,N-dimethyldiguanide) twice a day, were seen at weekly intervals, and the dose increased by 500 mg until either a dose was reached of 1.0 gm twice a day or fasting plasma glucose concentrations were less than 7 mmol/L. Once either of these end points had been achieved, patients were followed for an additional 10 weeks until they were re-admitted to the GCRC to repeat all the baseline measurements. It shold be noted that there were no changes in either blood count or chemical screening battery following metformin treatment. Results are expressed as mean ± SEM and the statistical significance of difference between the 2 groups assessed by Student's paired *t* test.

RESULTS

Table 1 lists relevant anthropometric and metabolic variables before and after the treatment period. It can be seen from these data that the only significant change seen following treatment with metformin was the improvement in glycemic control. Furthermore, there were no changes in the results of the chemical screening battery in either group.

Figure 1 illustrates the changes in fasting plasma glucose and ADMA concentrations associated with metformin treatment. As seen in Table 1, the data in Fig 1A show that fasting plasma glucose concentrations decreased to a similar degree, irrespective of whether patients received metfomin as monotherapy or added to a sulfonylurea compound.

ADMA concentrations before and after the administration of metformin are depicted in Fig 1B. These results demonstrate that metformin treatment was associated with significant decreases in plasma ADMA concentrations ($P < .01$), and that the decrease was comparable when metformin was administered to patients with type 2 diabetes by itself (before 1.65 ± 0.21, after 1.18 ± 0.13 μmol/L) or in combination with a

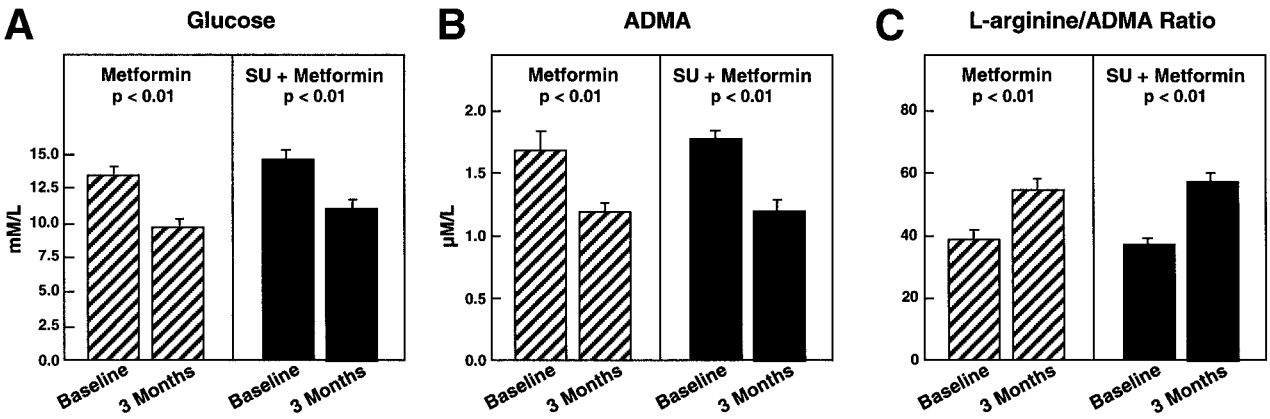

Fig 1. (A) Comparison of plasma glucose, (B) ADMA concentrations, and (C) L-arginine/ADMA ratio at baseline and 3 months after administration of metformin by itself or in combination with sulfonylurea.

sulfonylurea compound (before 1.75 ± 0.13, after 1.19 ± 0.08 μmol/L). In contrast, there was no difference in SDMA concentrations seen in either group. Similarly, there was no associated change in plasma L-arginine levels (metformin, before 71 ± 4, after 73 ± 4; sulfonylurea + metformin, before 67 ± 5, after 71 ± 5 μmol/L), resulting in a favorable increase in L-arginine/ADMA ratios in the metformin-treated individuals (Fig 1C).

Finally, it should be noted that there was no relationship between the decrease in ADMA concentrations and either the improvement in glycemic control or renal function as estimated by plasma creatinine concentration.

DISCUSSION

Reducing the incidence of CHD in patients with type 2 diabetes remains an unsolved conundrum, and it is unlikely that a simple cause and effect relationship will ever be discerned.[2,11] The results presented certainly offer no simple solution to this unresolved dilemma, but they clearly provide evidence that lowering plasma glucose concentration with metformin in patients with poor glycemic control is associated with a significant decrease in plasma ADMA concentration. We have previously demonstrated that plasma ADMA concentrations are 2-fold higher in untreated patients with type 2 diabetes compared with a matched control population (1.59 ± 0.22 *v* 0.69 ± 0.04 μmol/L).[8] These elevations were similar to individuals with other known risk factors, such as hypercholesterolemia and hypertension[5,7] and are likely to have pathophysiologic consequences. Moreover, since vascular cells are thought to be a major source of ADMA, the doubling of plasma concentrations may reflect an even greater change within endothelial cells.[12]

Faraci et al[13] demonstrated the dissociation constant of the enzyme/inhibitor complex (Ki) of NOS derived from cerebellum to be 2.8 μmol/L, and that concentrations as low as 1 μmol/L are able to induce contractions of cerebral vessels. Furthermore, we have previously demonstrated that ADMA concentrations similar to those found in the plasma of diabetic and hypercholesterolemic individuals had proinflammatory effects upon cultured endothelial cells.[14,15] Important to endothelial function, the elevation in ADMA concentrations coupled with little alteration in plasma L-arginine levels results in depressed L-arginine/ADMA ratios. Indeed, L-arginine/ADMA ratios equal to those found in diabetic individuals caused reduced NOS activity in animal models, as well as in humans.[5,16,17] Intravenous or oral administration of L-arginine to normalize L-arginine/ADMA ratios results in restoration of endothelial function. Therefore, it is likely that the reduction in ADMA by metformin treatment is likely to have equally beneficial results.

Our evidence that improved glycemic control with metformin also resulted in lowered plasma ADMA concentrations provides a possible explanation for the beneficial effect of insulin therapy on endothelial function, ie, a decrease in plasma ADMA concentrations. This explanation is obviously speculative and requires experimental validation. However, it is not inconsistent with the known potency of ADMA to inhibit NOS and endothelial function.[3,13]

Although it is clear that metformin treatement led to both an improvement in glycemic control and a decrease in plasma ADMA concentrations, it is not clear how much of the change in plasma ADMA concentration was secondary to the decrease in plasma glucose concentration, as contrasted to a direct effect of metformin. In this context, it is worth noting that we were not able to discern a significant relationship between the improvement in glycemic control and the decrease in plasma ADMA concentration. On the other hand, the goal of our study was to evaluate the effect of a specific antihyperglycemic compound on plasma ADMA concentration, not to address the more general question of the relationship between glycemia and plasma ADMA concentration.

Finally, our results do not help resolve the apparent paradox related to metformin treatment of overweight patients noted in the UKPDS.[1] Our results simply add to previous evidence[9,18] that the therapeutic effects of metformin are similar when used by itself or in combination with a sulfonylurea.

In conclusion, the results of this study provide the first evidence that plasma ADMA concentrations can be attenuated by clinically relevant antihyperglycemic treatment. Whether this effect is specific to metformin remains to be evaluated. However, since decreases in NO concentration and/or effectiveness lead to a variety of changes that impair endothelial function and enhance the atherogenic process in patients with type 2 diabetes, changes in plasma ADMA concentration, by regulating the activity of NOS,[9] have the capability of playing a central role in the regulation of endothelial function. The results of this study provide evidence that plasma ADMA concentrations can be modulated by therapeutic interventions in a syndrome associated with abnormal endothelial function.

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