Inhibition of L-Arginine Metabolizing Enzymes by L-Arginine-Derived Advanced Glycation End Products

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Summary Nω-Carboxymethyl-arginine (CMA), Nω-carboxyethyl-arginine (CEA) and Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) have been identified as L-arginine-derived advanced glycation end products (AGEs) formed by non-enzymatic reactions between reducing sugars such as glucose and amino groups in proteins. These AGEs are structurally analogous to endogenous inhibitors of nitric oxide synthases (NOS) including N⁶-monomethyl-L-arginine (L-NMMA) and asymmetric N⁶,N⁶'-dimethyl-L-arginine (ADMA). Increased plasma levels of these NOS inhibitors, and thus impaired generation of NO in vivo has been associated with the pathogenesis of vascular complications such as kidney failure and atherosclerosis. For these reasons we examined whether L-arginine-derived AGEs inhibit the activities of three L-arginine metabolizing enzymes including three isoforms of NOS (endothelium, neuronal and inducible NOS), dimethylarginine dimethylaminohydrolase (DDAH) that catalyzes the hydrolytic degradation of L-NMMA and ADMA to L-citrulline, and arginase that modulates intracellular L-arginine bioavailability. We found that AGEs inhibited the in vitro activities of endothelium type NOS weakly (IC₅₀ values of CMA, CEA and MG-H1 were 830, 3870 and 1280 µM, respectively) and were also potential endogenous inhibitors for arginase (IC₅₀ values of CMA and CML were 1470 and 1060 µM), but were poor inhibitors for DDAH. These results suggest that the tested L-arginine- and L-lysine-derived AGEs appear not to impair NO biosynthesis directly.

Key Words: advanced glycation end products, Nω-carboxymethyl-arginine, nitric oxide synthase, dimethylarginine dimethylaminohydrolase, arginase

Introduction

Advanced glycation end products (AGEs) are a group of post-translationally modified proteins which are formed from the reactions between amino residues and reducing sugars such as glucose. Levels of glycated proteins increase with aging under normal physiological conditions and alter the molecular structure and biochemical functions of the affected proteins. Hyperglycemia accelerates the glycation and leads to excessive accumulation of AGEs-modified proteins in vessel walls and has been linked to the pathogenesis of diabetic complications, particularly the development of micro- and macrovascular diseases [1, 2]. Previous studies have shown that AGEs-modified proteins quench nitric oxide (NO), an endothelium-derived vasodilator, in vitro [3, 4] and also decrease the enzymatic activities and protein expression of endothelium NO synthase (eNOS) in...
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vascular endothelium cell cultures [5–7]. These results suggest that AGEs impair NO biosynthesis, playing a critical role in diabetic endothelial dysfunction.

The reactive dicarbonyl intermediates (glyoxal and methylglyoxal) that are generated from auto-oxidation of glucose and the glycolysis pathway [8, 9] rapidly modifies the amino groups of arginine and lysine residues. Some glycated arginine derivatives have been structurally characterized as \(N^\omega\)-carboxymethylarginine (CMA) [10], \(N^\omega\)-carboxyethylarginine (CEA) [11] and \(N^\delta\)-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) [12] (see structures in Fig. 1). Several previous studies have shown that the levels of glycated arginine derivatives are markedly elevated in the plasma of patients with diabetic complications compared with those in healthy individuals [13]. In addition, immunohistochemical studies have demonstrated that CMA accumulates in the atherosclerotic lesions of human aorta, the CMA levels in tissue proteins being positively correlated with the pathogenesis of atherosclerosis [14].

NO, identified as an endothelium-derived relaxing factor (EDRF), is a critical signaling molecule in vascular function and homeostasis in mammals [15]. NO is formed during the oxidation of L-arginine to L-citrulline that is catalyzed by NO synthases (NOSs, EC 1.14.13.39). Three NOS isoforms have been characterized and cloned: neuronal NOS (nNOS; type I) and inducible NOS (iNOS; type II), endothelial NOS (eNOS; type III) [16]. Constitutive NOS (eNOS and nNOS) are associated with the regulation of vascular tone in blood vessels and neurotransmission in the central and peripheral nervous tissues. In the immune system, inflammatory cytokines stimulate iNOS expression in macrophages and other cell types to provide defense against pathogens.

\(N^N\)-Monomethyl-L-arginine (L-NMMA) and \(N^N, \ N^\omega\)-dimethyl-L-arginine (asymmetric dimethylarginine; ADMA) are L-arginine analogues and typical endogenous NOSs inhibitors (see structures in Fig. 1). It has been demonstrated that levels of these methylarginines are elevated in plasma and tissues of patients with various pathological conditions, including hypertension [17], chronic renal failure [18] and

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**Fig. 1.** Structures of L-arginine-derived AGEs and other compounds used in this study. (a) and (b) endogenous NOS inhibitor, L-NMMA and ADMA, respectively; (c)–(e) L-arginine-derived AGEs, CMA, CEA and MG-H1; (f) L-arginine; (g) L-lysine-derived AGEs, CML; (h) an intermediate metabolite in the L-arginine-NO biosynthesis pathway, NOHA; (i) nor-NOHA, a synthetic analog of NOHA. Dotted circles indicate variable chemical groups of L-arginine derivatives.
diabetes mellitus [19], resulting in a deficiency of endogenous biosynthesis of NO due to inhibition of NOSs, especially eNOS.

Methylarginines are generated by the methylation of L-arginine residues in proteins mediated with S-adenosylmethionine: protein arginine methyltransferases (protein methylases). The methyl groups are symmetrically or asymmetrically-adducted to the guanido group of L-arginine, resulting in formation of L-NMMA and AMDA (see structures in Fig. 1). Endogenous methylarginines are in part excreted by the kidney. However the metabolism of L-NMMA and ADMA, but not \( \text{N}^2,\text{N}^\text{O} \)-dimethyl-L-arginine (symmetric dimethylarginine, SDMA), mainly occurs via hydrolytic degradation to L-citrulline and either monomethylamine or dimethylamine by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) [20]. Inhibition of DDAH activity increases intracellular concentrations of ADMA and reduces endogenous NO generation [21]. It has been reported that a glucose-induced impairment of DDAH causes the accumulation of ADMA and this may contribute to endothelial vasodilator dysfunction in patients with diabetes [22].

The NO generation depends on the bioavailability of NO\( \text{s} \) substrate L-arginine which is shared by arginase, the enzyme metabolizing L-arginine to form urea and L-ornithine via the urea cycle [23]. Arginase is responsible for regulating NO generation by modulating intracellular L-arginine bioavailability. It has been reported that in arginase activity diminish plasma levels of L-arginine in human diabetes patients and experimental diabetic animals [24, 25].

The present study has focused on several endogenous AGEs-derived compounds including L-arginine derivatives (CMA, CEA and MG-H1) and an L-lysine derivative (carboxymethyl-lysine, CML). Because these compounds are structurally analogous to endogenous NO\( \text{s} \) inhibitors L-NMMA and ADMA, they may be implicated in the pathological mechanism of chronic vasculopathy through competitive inhibition of NO\( \text{s} \) activities, especially eNOS. These L-arginine derivatives may impair NO generation by antagonizing L-arginine binding sites of three NOS isoforms. In addition to NOS we have also examined the effects of these compounds on the enzymatic activities of DDAH and arginase, both enzymes indirectly regulating NO bioavailability.

Materials and Methods

Materials

L-Arginine, ADMA, \( \text{N}^\text{O} \)-hydroxy-L-arginine (NOHA), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), (6R)-5,6,7,8-tetrahydrobiopterin (BH4), recombinant eNOS (from bovine), iNOS (from mouse macrophage), Dowex® AG50W-X8 cation-exchange resin (mesh size, 100–200) were all purchased from Sigma Chemicals (St. Louis, MO). Ultima Gold XR scintillation liquid was from PerkinElmer (Shelton, CA). Recombinant nNOS (from rat) and \( \text{N}^\text{O} \)-hydroxy-nor-arginine (nor-NOHA) were obtained from Cayman Chemicals. Arginase (from bovine liver) was from Alexis Biochemicals (Lausen, Switzerland). L-NMMA was from Calbiochem, Darmstadt, Germany. All other chemicals were obtained from Wako Pure Chemical Industries, Osaka, Japan.

Synthesis of L-arginine-derived AGEs

CMA, CEA and MG-H1 were synthesized and purified according to the methods described previously [26]. Their structures were confirmed by nuclear magnetic resonance and electrospray ion mass spectrometry. Purities of these L-arginine derivatives were over 98%, which was verified by high performance liquid chromatography equipped with a fluorescence detector after derivatization with the fluorescent reagent 4-fluoro-7-nitro-2,1,3-benzoxadiazole [27].

Assay for NOS\( \text{s} \) activities

The NOS\( \text{s} \) activities were determined by monitoring the conversion of L-\( \text{[}^{14}\text{C(U)}\text{-}] \)-arginine (Moravek, Brea, CA) to L-\( \text{[}^{14}\text{C(U)}\text{-}] \)-citrulline as previously described [28]. Recombinant enzymes of three isoforms of NOS (specific activity: eNOS 0.03, nNOS 2.9, iNOS 0.62 units/mg protein) (10 \( \mu \)l) were added to a reaction mixture (final volume of 100 \( \mu \)l) consisting of 50 mM HEPES buffer (pH 7.4), 1 mM NADPH, 4 \( \mu \)M FAD, 4 \( \mu \)M FMN, 10 \( \mu \)M BH4, 1 mg/L calmodulin, 2.5 mM CaCl₂, 50 \( \mu \)M L-arginine and 0.5 \( \mu \)Ci/ml of L-\( \text{[}^{14}\text{C(U)}\text{-}] \)-arginine (specific activity: 8.14–11.1 GBq/mmol). After incubation for 30 min at 37°C, the reactions were terminated by addition of 0.5 ml of 20 mM HEPES (pH 5.5) containing 2 mM EDTA and 2 mM EGTA. Samples were passed through a 1 ml column of Dowex AG50W-X8 (Na⁺-form) to remove unconverted L-\( \text{[}^{14}\text{C(U)}\text{-}] \)-arginine. L-\( \text{[}^{14}\text{C(U)}\text{-}] \)-Citrulline was eluted twice with 1 ml of distilled water, and the eluted fractions mixed with 10 ml of cocktail (Ultima Gold XR) were quantified using a liquid scintillation counter. A reaction mixture containing L-\( \text{[}^{14}\text{C(U)}\text{-}] \)-arginine without the enzyme was incubated as a control to determine background counts. The effects of arginine derivatives on the NO\( \text{s} \) activities were examined in the presence and absence of the test compounds at various concentrations.

Assay for arginase activity

Assays for arginase activity were performed by monitoring the conversion of L-\( \text{[}^{14}\text{C(U)}\text{-}] \)-arginine (Moravek, Brea, CA) to \( \text{[}^{14}\text{C]} \)-urea as previously described [28]. Recombinant arginase (0.5 \( \mu \)g of protein, specific activity: 0.094 units/mg protein) (10 \( \mu \)l) was added to a mixture (final
volume of 100 µl) containing 10 mM Tris-HCl buffer, pH 9.6, 1 mM MnCl₂, and 0.16 µCi/ml of L-[guanidine-¹⁴C]-arginine (specific activity: 1.85–2.22 GBq/mmol). After incubation for 1 h at 37°C, the reactions were terminated by addition of 0.4 ml of stop buffer containing 250 mM sodium acetate and 100 mM urea, pH 4.5. Samples were then passed through a 1 ml column of Dowex AG50W-8X (Na⁺-form) to remove unmetabolized L-[guanidine-¹⁴C]-arginine. [¹⁴C]-Urea was eluted twice with 1 ml of distilled water, and the eluted fractions mixed with 10 ml of cocktail (Ultima Gold XR) were quantified using a liquid scintillation counter. A reaction mixture containing L-[guanidine-¹⁴C]-arginine in the absence of enzyme was incubated as a control to determine background counts. The inhibitory effects of arginase were examined in the presence and absence of the test compounds at various concentrations.

**Assay for dimethylarginine dimethylaminohydrolase (DDAH) activity**

The DDAH activity was determined by monitoring the conversion of L-[4,5-³H]-NMMA (American Radiolabeled Chemicals, Inc., St. Louis, MO) to L-[³H]-citrulline as previously described [28, 29]. Rat kidney tissues (0.5 g) were homogenized in 1 ml of ice-cold 0.1 M sodium phosphate buffer (SPB, pH 6.5), and then centrifuged at 10,000 rpm for 5 min at 4°C. The supernatants were analyzed for protein content using a BCA protein reagent kit with bovine serum albumin as the standard. The supernatants were diluted with SPB to 1 mg protein in 50 µl, and then were added to a reaction mixture (a final volume of 100 µl) containing 0.1 M SPB (pH 6.5) and 0.1 µCi/ml of L-[³H]-NMMA (specific activity: 1.48–2.96 TBq/mmol). After incubation for 1 h at 37°C, the reactions were terminated by keeping the tubes on ice for 5 min. Samples were then passed through a 1 ml column of Dowex AG50W-8X (Na⁺-form) to remove unmetabolized L-[³H]-NMMA. L-[³H]-Citrulline was eluted twice with 1 ml of distilled water, and the eluted fractions mixed with 10 ml of cocktail (Ultima Gold XR) were quantified using a liquid scintillation counter. A reaction mixture containing L-[³H]-NMMA in the absence of enzyme was added to the Dowex column to determine background counts. The effects of arginine derivatives on the enzyme activities in the presence and absence of the test compounds at various concentrations were examined.

**Statistical analysis**

All experiments were repeated 3–8 times. Representative results are shown as means ± standard deviations (SD). For statistical comparison between two groups, an unpaired t-test was used.
Effects of AGEs on L-Arginine Metabolizing Enzymes

Table 1. The IC₅₀ values of various arginine derivatives for inhibition of three isoforms of NOS.

| Compounds | eNOS (μM) | iNOS (μM) | nNOS (μM) |
|-----------|-----------|-----------|-----------|
| L-NMMA    | 5 ± 1     | 10 ± 3    | 8 ± 2     |
| ADMA      | 16 ± 9    | 24 ± 1    | 23 ± 0    |
| CMA       | 830 ± 36  | 1160 ± 40 | >5000     |
| CEA       | 3870 ± 680| >5000     | >5000     |
| MG-H1     | 1280 ± 75 | 2970 ± 560| 4170 ± 380|
| CML       | >5000     | >5000     | 1930 ± 300|

The effects of various L-arginine derivatives on the activities of three isoforms of NOSs were measured as described in Materials and Methods. The values correspond to concentrations necessary for 50% inhibition of enzyme activities. Results are means ± SD (n = 4).

Results

Effects of L-arginine-derived AGEs on activities of three NOS isoforms

The effects of four AGEs compounds (CMA, CEA, MG-H1 and CML) on the activities of three NOS isoforms were examined at final concentrations of 5, 50, 500, 2500, and 5000 μM. The conversion of L-[¹⁴C(U)]-arginine to [¹⁴C(U)]-citrulline by NOS isoforms was decreased in a concentration-dependent manner when the enzymatic reaction was carried out in the presence of these AGEs. In particular, the eNOS activity was efficiently inhibited by CMA (IC₅₀ value of 830 ± 36 μM) and MG-H1 (IC₅₀ value of 1280 ± 75 μM), but not by CEA (IC₅₀ value of 3870 ± 680 μM) and CML, a glycated L-lysine derivative (IC₅₀ value >5000 μM) (Fig. 2A, Table 1). On the other hand, L-NMMA and ADMA, well known inhibitors for NOSs, inhibited the activities of eNOS strongly with IC₅₀ values being 5 ± 1 and 16 ± 9 μM, respectively. Similar to eNOS, the iNOS activity was also inhibited by CMA (1160 ± 40 μM), MG-H1 (2970 ± 560 μM), but not by CEA and CML (both IC₅₀ values of >5000 μM) whereas L-NMMA and ADMA inhibited iNOS activities with IC₅₀ values of 10 ± 3 and 24 ± 1 μM, respectively (Fig. 2B, Table 1). The nNOS activity was moderately inhibited by CML (1930 ± 300 μM), MG-H1 (4170 ± 380 μM), and very little by CMA and CEA (with IC₅₀ values of >5000 μM (Fig. 2C, Table 1). L-NMMA and ADMA inhibited the activities of nNOS with IC₅₀ values being 8 ± 2 and 23 ± 0 μM, respectively.

Effects of L-arginine-derived AGEs on activities of arginase and DDAH

The metabolism of L-[guanidine-¹⁴C]-arginine to [¹⁴C]-urea mediated by arginase was diminished by the presence of AGEs compounds. In particular, CML suppressed the formation of [¹⁴C]-urea in a dose-dependent manner with an IC₅₀ value of 1060 ± 60 μM, whereas CMA inhibited the arginase activity with an IC₅₀ value of 1470 ± 120 μM. CEA and MG-H1 showed very little inhibition at the concentrations tested with IC₅₀ >5000 μM (Fig. 3A, Table 2). NOHA, an intermediate metabolite in the L-arginine-NO biosynthesis pathway, and nor-NOHA, a synthetic analog of NOHA, are known inhibitors for arginase [30] and efficiently inhibited the activity of arginase in a dose-dependent manner, with the IC₅₀ values being 230 ± 26 and 340 ± 12 μM, respectively (Fig. 3A, Table 2).

The enzymatic activity of DDAH was assayed by counting the amounts of L-[¹³C]-citrulline catalytically formed from L-[¹³C]-NMMA by a homogenate of rat kidney tissue [28, 29]. AGEs (CMA, CEA, MG-H1 and CML) were found to exhibit weak inhibitory effects on DDAH activity with IC₅₀ values being >5000 μM for each compound (Fig. 3B, Table 2). We have also examined the inhibitory effect of nor-NOHA, a structurally-analogous compound to a potent DDAH inhibitor S-2-amino-4(5-methylguanidino) butanoic acid (4124W) [27], nor-NOHA inhibited DDAH activity with an IC₅₀ value of 1770 ± 250 μM.

Discussion

We have examined our hypothesis that L-arginine-derived AGEs, CMA, CEA and MG-H1 may inhibit L-arginine metabolism such as the biosyntheses of NO and urea, and contribute to the pathogenesis of vascular complications such as kidney failure and atherosclerosis in diabetes patients. L-Arginine-derived AGEs are structurally analogous to the endogenous NOS inhibitor methylarginine and their levels have been reported to be markedly elevated in the plasma of patients with diabetes compared with those in healthy individuals [13, 31]. For these reasons we have synthesized CMA, CEA and MG-H1 and examined their effects on the enzymatic activities of three isoforms of NOS as well as DDAH and arginase that are enzymes which indirectly regulate NO bioavailability.

We found that glycated L-arginine derivatives (CMA, CEA and MG-H1) inhibited all of three NOS isoforms.
(eNOS, nNOS, and iNOS) as well as arginase, whereas they did not show inhibitory effects on DDAH activity. The glycated L-lysine derivative (CML) inhibited arginase, but not NOSs and DDAH. However, the IC₅₀ values of these L-arginine derivatives were 3 to 1000-fold higher than those of the synthetic endogenous NOS inhibitors such as ADMA and NOHA.

Reactive α-oxoaldehydes of glyoxal and methylglyoxal (the major AGEs precursors) react with L-arginine and L-lysine residues in protein to form AGEs such as CMA, CEA, MG-H1 and CML. AGEs-modified proteins accumulate in tissues age-dependently and their accumulation is increased under some pathophysiological conditions. Thornalley et al. [31] determined the concentrations of 12 AGEs including MG-H1 and CML by liquid chromatography-mass spectrometry (LC-MS) after enzymatic hydrolysis and found significantly increased levels of AGEs residues in plasma proteins (up to 7-fold) in patients with renal failure compared to those in normal healthy subjects. Similarly the concentrations of CMA residues in serum protein analyzed by LC-MS were ~1.5-fold higher in patients with diabetes without renal failure than in normal subjects [13]. In addition, the levels of free MG-H1 and CML were reported to be 0.11 μM and 0.023 μM in plasma of normal individuals, whereas their levels rose to approximately 5.5 μM and 0.2 μM, respectively, in patients with end-stage renal disease [31].

Under our in vitro assay conditions, these AGEs compounds inhibited NOS activities with IC₅₀ values being >830 μM. It is therefore unlikely that glycated L-arginine and L-lysine derivatives such as CMA, CEA, MG-H1 and CML competitively inhibit the enzymatic activities of the three isoforms of NOS.

Many studies have demonstrated that elevated plasma levels of ADMA might contribute at least in part to the molecular mechanisms of vasculopathy by impairment of NO-dependent vasodilation under various pathophysiological states [17–19]. The plasma concentrations of ADMA found in healthy populations are 0.5 ~ 1.2 μM, whereas they increase up to 10-fold in patients with end-stage renal disease and more moderately (2–3 fold) in many other disease states including chronic heart failure and hypercholesterolemia [32, 33].

Free methylarginines, derived from the degradation of methylated proteins and more than 90% of L-NMMA and ADMA, but not symmetric dimethylarginine (SDMA), are

| Compounds    | Arginase IC₅₀ (μM) | DDAH IC₅₀ (μM) |
|--------------|--------------------|----------------|
| Nor-NOHA     | 230 ± 26           | 1770 ± 250     |
| NOHA         | 340 ± 12           | ND             |
| CMA          | 1470 ± 120         | >5000          |
| CEA          | >5000              | 5000           |
| MG-H1        | >5000              | 5000           |
| CML          | 1060 ± 60          | >5000          |

The effects of various L-arginine derivatives on the activities of arginase and DDAH were measured as described in Materials and Methods. The values correspond to concentrations necessary for 50% inhibition of enzyme activities. Results are means ± SD (n = 4). ND: not determined.
mainly metabolized by the enzyme DDAH, which catalyzes the degradation of L-NMMA and ADMA to form L-citrulline and monomethylamine or dimethylamine, respectively. DDAH expression is found in many tissues (endothelial cells, brain etc.), but the liver and kidney are the primary organs responsible for the metabolism of L-NMMA and ADMA [34]. The concentrations of endogenous NOS inhibitors modulated by DDAH are associated with L-arginine/NO-dependent vasodilation. Furthermore recent studies suggest that ADMA accumulation caused by an impairment of DDAH due to down-regulation of protein expression and its decreased activity contributes to abnormal endothelium-dependent vasodilation in diabetes mellitus [22, 35]. For these reasons, we have investigated the effects of AGEs (CMA, CEA, MG-H1 and CML) on the enzymatic activities of DDAH. The DDAH activity was slightly inhibited by the test compounds (CMA, MG-H1 and CML) with an IC$_{50}$ value being >5000 μM for each compound (Fig. 3B, Table 2). Even in the presence of the highest concentrations (5000 μM) of these compounds, the DDAH activity was inhibited only by approximately 60%. Because the DDAH inhibitor 4124W [21] could not be obtained commercially, we examined the effect of nor-NOHA, a structural analogue to 4124W, on the DDAH activity. We found that nor-NOHA showed a dose-dependent inhibition of the conversion of L-[^3]H-NMMA to L-[^3]H-citrulline with an IC$_{50}$ value of 1770 μM. Compared with nor-NOHA, AGEs exhibited weaker inhibitory effects on the DDAH activity.

The generation of vasodilator (NO) depends also on the bioavailability of the NOS substrate, L-arginine, which is shared by an enzyme arginase forming urea and L-ornithine via the urea cycle [36]. It has been reported that an increased activity of arginase causes diminished levels of plasma L-arginine in human diabetes patients and experimental diabetic animals [24, 25]. For these reasons, we examined the effects of AGEs compounds on the arginase activity. CML and CMA (IC$_{50}$ values of 1060 and 1470 μM, respectively), but not CEA and MG-H1 (IC$_{50}$ values >5000 μM), were found to be moderate inhibitors of arginase, although the inhibitory efficiencies were approximately 3-fold weaker than a well known arginase inhibitor, NOHA (IC$_{50}$ value 340 μM), that is an intermediate of the L-arginine/NO pathway.

In conclusion, the present study demonstrated that glycated L-arginine derivatives (CMA, CEA, MG-H1 and CML) and an L-lysine derivative (CML) are potential endogenous inhibitors for three NOS isoforms (eNOS, nNOS, and iNOS) and arginase, but are very poor inhibitors for DDAH. The IC$_{50}$ values were much greater than the well-known endogenous NOSSs and arginase inhibitors such as ADMA and NOHA. Further studies are needed to determine whether AGEs (CMA, CEA, MG-H1 and CML) inhibit other arginine metabolizing enzymes such as argininosuccinate synthase and argininosuccinate lyase and also affect L-arginine transport into cells, which is also known to play a role in the regulation of NO production by cells.

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**Abbreviations**

AGEs, Advanced glycation end products; ADMA, asymmetric N$^\beta$,N$^\alpha$-dimethyl-L-arginine; CMA, carboxymethyl-arginine; CEA, carboxyethyl-arginine; CML, carboxymethyllysine; DDAH, dimethylarginine dimethylaminohydrolase; EDRF, endothelium-derived relaxing factor; MG-H1, N$^\alpha$-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; NOHA, N$^\alpha$-hydroxy-L-arginine; nor-NOHA, N$^\alpha$-hydroxy-nor-arginine; LC-MS, liquid chromatography-mass spectrometry; L-NMMA, N$^\alpha$-monomethyl-L-arginine; NOS, NO synthase; nNOS, neuronal NOS; iNOS, inducible NOS; eNOS, endothelial NOS; SDMA, symmetric N$^\alpha$,N$^\gamma$-dimethyl-L-arginine; SPB, sodium phosphate buffer; BH4, (6R)-5,6,7,8-tetrahydrobiopterin.

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