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Original article

Reduction in advanced glycation end products by ACE inhibitor in diabetic cardiomyopathy model

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Background: Advanced glycation end-products (AGEs) due to hyperglycemia have been reported with diabetic complications. The effect of angiotensin converting enzyme (ACE) inhibition on the formation of AGEs and oxidative stress in the myocardium was explored.
Methods: Streptozocin-induced diabetic rats were randomized to three groups including untreated, treated with ACE inhibitor captopril, and treated with aminoguanidine for 24 weeks with non-diabetic rats as control.
Results: At study end, elevations of blood pressure, heart to body weight ratio, and brain natriuretic peptide levels were found in diabetic rats, indicating cardiac hypertrophy and dysfunction. Accumulation of myocardial AGEs/ receptor of AGEs (RAGEs), as determined by immunohistochemistry and Western blots were increased in diabetic animals, which were attenuated by both captopril and aminoguanidine. Staining of nitrotyrosine and 8-hydroxydeaminoguanosine, markers of oxidative stress, also increased in diabetic rats and was attenuated by both captopril and aminoguanidine treatment. The myocardial pentosidine, a marker of AGEs, increased in diabetic rats but was not significantly affected by either treatment.
Conclusion: This study has identified a relationship between the renin-angiotensin system and the accumulation of AGEs in experimental diabetic hearts that may be linked through oxidative stress.

Keyword: ACE inhibitor; advanced glycation end products; aminoguanidine; diabetic mellitus; oxidative stress.

List of Abbreviations
ACE = angiotensin converting enzyme
AGE =: advanced glycation end-products
CML = carboxymethyllysine
RAGES = receptor of AGEs
RAS = renin-angiotensin system
ROS = reactive oxygen species
SBP = systolic blood pressure

Diabetes mellitus leads to cardiovascular morbidity and mortality because of functional and morphological damage [1, 2], and affects cardiac structure and function independently of blood pressure or coronary artery disease [3]. Diabetic cardiomyopathy is characterized by microvascular pathology and interstitial fibrosis [4] and is associated with the duration and severity of hyperglycemia [3]. Chronic hyperglycemia facilitates the Maillard reaction, a non-enzymatic reaction between reducing sugars and free reactive amino groups of proteins [5]. Subsequent biochemical modifications result in the irreversible formation of a heterogeneous group of products known as advanced glycation end-products (AGEs). AGEs are known to contribute to the onset of diabetic cardiomyopathy [6], and inhibition of AGE formation by treatment with the hydrazine compound aminoguanidine in experimental diabetes has resulted in beneficial effects on retinopathy, nephropathy, and neuropathy [7-9].

A well-known pathogenic mechanism of diabetes is excessive oxidative stress arising from an imbalance between generation and elimination of

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reactive oxygen species (ROS) at the cellular level [10]. Increased synthesis of AGEs is one source of ROS in diabetes [11-13].

Angiotensin II, the major hormone of the renin-angiotensin system (RAS), has a direct effect on end organs, including the heart in addition to its effect on blood vessels [14]. Abnormal production of angiotensin II is associated with elevated cellular oxidative stress and high rates of apoptotic cell death in humans and in animal models of diabetes. Recent clinical trials have consistently shown that pharmacological blockage of the RAS reduces the development of cardiovascular morbidity and the risk of death in diabetic patients [15, 16]. Interruption of the RAS with an angiotensin converting enzyme (ACE) inhibitor has proven beneficial in diabetic renal disease [17] and in advanced congestive heart failure [18]. The benefits have been attributed not only to interruption of hemodynamic effects but more recently also to the non-hemodynamic effects of angiotensin II [15, 16, 19]. While diabetes is established as a risk for heart failure, the pathophysiological relationships between diabetes, AGEs, and the RAS are not completely understood. The objective of the present study was to assess the influence of AGEs in diabetic hearts and whether the inhibition of the RAS with an ACE inhibitor would reduce accumulation of AGEs, levels of oxidative stress, and the magnitude of myocardial fibrosis in a rat model of diabetes.

Materials and Methods

Animals

Male Sprague Dawley rats (200 to 250 g) were fasted overnight. Experimental diabetes was induced by an intra-peritoneal injection of streptozocin (50 mg/kg) (STZ, Sigma Chemical, St. Louis, MO) dissolved in sodium citrate saline buffer (pH 4.5). Animals with plasma glucose concentration above 180 mg/dl 7 days after induction were included in the study as diabetic. Rats sham-injected with vehicle served as non-diabetic control group (n = 8, group C). Diabetic rats were randomly assigned to 3 groups, including rats without treatment (n = 8, group D), rats administered with captopril (Bristol-Myers Squibb) 25 mg/kg/day in drinking water (n = 8, group DC), and rats administered with aminoguanidine (Fluka Chemica, Buchs, Switzerland) 1g/L in drinking water (n = 8, group DA). All rats were treated for 24 weeks. Water consumption was monitored 3 times a week in order to adjust the concentrations of captopril.

Body weight was monitored weekly. Before sacrifice at the end of the study, heart rate (HR) and systolic blood pressure (SBP) were measured non-invasively in conscious rats by the tail-cuff method (MK-2000ST, Muromachi Kakai Co., Ltd.). Blood was collected from tail vein and blood glucose was assayed with a glucometer (One Touch Profile, LifeScan, Inc.) at the end of study before sacrifice. Blood samples were collected from the inferior vena cava for brain natriuretic peptide ELISA (Assaypro LLC.).

Procedures involving animal use and their care were approved and conducted in accordance with the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital.

Tissue preparation

At sacrifice, the heart was excised from the chest, rinsed with ice-cold phosphate-buffered saline (PBS), gently blotted, and weighed. Paraffin-embedded sections were then prepared from mid-ventricular sections of the left ventricle.

Immunohistochemistry

A modified ABC immunoglobulin enzyme bridge technique [20] was used for immunohistochemistry. Formalin-fixed paraffin sections of heart were dewaxed and hydrated. After incubation with 0.3% hydrogen peroxide for 20 minutes, sections were incubated with protein-blocking agent (Lipshaw, Pittsburgh, PA) for 30 minutes and then with monoclonal primary antibody against AGEs (COSMO BIO, recognizing primarily carboxymethyllysine (CML) (19)) or RAGEs (Chemicon International, Temecula, CA, USA) for 1 h. Pilot studies were performed to determine the specificity and the optimal concentrations of the ant-CML and anti-RAGEs antibodies. Tissue sections were then stained with biotinylated secondary antibody for 10 minutes and avidin-biotin horseradish peroxidase complex for 15 minutes using Vectastain ABC ELITE kit (Vector Laboratories, Burlingame, CA) before a substrate solution of 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical, St Louis, MO) was added. Sections were counterstained in hematoxylin and mounted.

In the negative control sections, the primary antibody was omitted. Diabetic arterial wall were included as positive control. Quantification of myocardial CML and RAGEs was performed by

measuring the proportional area of positive staining. Ten fields (x200) were counted per section. Histological sections of paraffin-embedded ventricle were also stained with Masson trichrome to quantify cardiac collagen deposition in each group. For this assay, the area of deposition on each slide was outlined at a magnification of 300x and quantified in duplicate by manual planimetry, with modification of the method described by Moreno et al [21]. Collagen deposition was used instead of hydroxyproline concentrations to assess fibrosis because it is more suitable for samples that have been stored frozen, as ours were. Furthermore, visualization of collagen deposition can give histological information as well as an estimation of the amount of fibrosis.

The number of cardiomyocyte nuclear profiles was counted in LV tissue section stained with hematoxylin and eosin. Under high power field (x400), fifteen fields were counted in the left ventricle to determine the number of nuclear profiles per unit area of myocardium.

Western blotting of AGEs, RAGES, nitrotyrosine, and pentosidine

Cardiac samples were homogenized in the lysis buffer containing protease inhibitors (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride [PMSF], and 1 μg/ml aprotonin). Cell debris was pelleted by centrifugation at 400 g and the supernatant further separated by ultracentrifugation at 75,000 g. The protein content of the second supernatant was determined using the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA), and equal amounts of proteins were separated by 10% SDS-PAGES (Biorad Laboratories, Richmond, CA) and transferred onto nitrocellulose membrane. The blots were blocked with 5% non-fat milk solution or, in the case of nitrotyrosine determination, with bovine serum albumin (BSA) and incubated with mouse antibodies against CML (1:2,000); pentosidine (1:1,500) (Cosmo Bio) RAGEs (1:1,500) or rabbit anti-nitrotyrosine (1:2000; Upstate Biotechnologies, Lake Placid, NY) overnight at 4°C with gentle rocking. After washing with 1 × Tris-buffered saline containing 0.05% Tween 20, blots were incubated with biotinylated secondary antibodies (1:15,000) (Dako, Carpinteria, Denmark) and then horseradish peroxidase-conjugated strepavidin (1:20,000) (Dako). Immunoreactivity was determined using ECL (Amersham Pharmacia Biotech, Buckinghamshire, U.K.). Band intensity was determined by computer-aided densitometry (Video Pro-32).

Nuclear reactive oxygen species detection in the left ventricle

The presence of oxidant hydroxyl radicals in myocyte nuclei was assessed by measuring 8-hydroxydeoxyguanosine (OHdG), a modified base that occurs in DNA during oxidative stress. After deparaffinization, formalin fixed LV sections were treated with proteinase K (50 μg/ml) in PBS for 60 minutes at 37°C prior to blocking with 1% BSA in PBS. Goat anti 8-OHdG polyclonal antibody (1:50) (Chemicon) was incubated overnight and revealed by DAB staining (1:100, Chemicon). 8-OHdG staining was quantified by counting the number of positive cardiomyocyte nuclei in 10 random fields (magnification 400×).

Statistical analysis

Continuous variables were compared by one-way analysis of variance (ANOVA). When a significant difference between groups was apparent, multiple comparisons of means were performed using the Bonferroni procedure with type-I error adjustment. Data are presented as mean SD. All statistical assessments were two-sided and evaluated at the 0.05 level of significant difference. Statistical analyses were performed using SPSS 15.0 statistics software (SPSS Inc, Chicago, IL).

Results

Metabolic and cardiovascular parameters of treated and untreated diabetic animals

Before sacrifice, all diabetic groups (D, DC and DA) showed a significant increase in blood glucose levels compared to the non-diabetic control group (D, DC and DA vs. C, p <0.01, **Table 1**). No differences in blood glucose levels were observed between treated (DC and DA) and untreated (D) diabetic groups.

In all diabetic groups, body weight was significantly lower than that of the non-diabetic control group irrespective of the presence or absence of drug treatment (p <0.01), and all diabetic groups also had a significant increase in the heart-to-body weight ratio compared to control (p <0.01). Untreated and aminoguanidine-treated, but not ACE inhibitor captopril -treated, diabetic rats had significant increases in systolic blood pressure compared to

nondiabetic controls (D vs. C and DA vs. C, p <0.01).

Serum brain natriuretic peptide, a biomarker of cardiac dysfunction, was significantly higher in the untreated diabetic rats than in the non-diabetic control rats (1.64±0.27 ng/ml vs. 0.18 0.04 ng/ml, p <0.001). Treatment with captopril significantly reduced the content of serum brain natriuretic peptide in diabetic rats, which is comparable to that in non-diabetic control. Although aminoguanidine treatment significantly reduced the content of serum brain natriuretic peptide in diabetic rats, the content of serum brain natriuretic peptide in diabetic rats was still significantly higher than that in non-diabetic control (**Table 1**). These data indicate that augmentation of serum brain natriuretic peptide in diabetic rats could be abolished by ACE inhibitor but only attenuated by AGE inhibitor.

Ultra-structural change and interstitial fibrosis

The number of left ventricular myocytes seen under high power field examination was significantly lower in the diabetic group than in the control group (69.4±8.2 vs.104.8±28.3, p <0.001, **Figure 1**). Treatment with captopril, but not aminoguanidine, significantly attenuated the myocyte loss seen in these diabetic animals (DC vs. D, p = 0.002; DA vs. D, p = 1.000). Diabetes was also accompanied by a significant accumulation in interstitial collagen (D vs. C, 38.9±7.2 vs. 8.9±2.2, p <0.001, **Figure 2**), which was significantly attenuated by both captopril and aminoguanidine treatment (p <0.01).

Table 1. Biochemical and metabolic parameters for rats at week 24

	C group (n = 8)	D group (n = 8)	DC group (n = 8)	DA group (n = 8)	P-value
Glucose (mg/dl)	78.1±10.5	283.6±18.9	306.9±41.8	290.4±17.1	<0.001*
Body weight (g)	465.1±31.9	324.8±21.7	313.1±17.5	312.9±19.1	<0.001*
Heart weight (g)	1.12±0.07	1.19±0.11	1.07±0.05	1.11±0.11	0.081
Heart to body weight ($\times 10^{-3}$)	2.43±0.27	3.65±0.23	3.34±0.16	3.54±0.39	<0.001*
SBP(mmHg)	104.0±12.6	138.4±15.9	117.1±8.4	137.3±13.2	<0.001*
Brain natriuretic peptide (ng/ml)	0.18±0.04	1.64±0.27	0.23±0.08	0.52±0.17	<0.001*

C = control, D = diabetic, DC = diabetic + captopril, DA = diabetic + aminoguanidine. Data are displayed as mean±standard deviation. *P*-values are based on ANOVA test. *p <0.05, †a significant difference between the indicated group and the control group, ‡a significant difference between the indicated group and the diabetic group. Pair-wise multiple comparisons between groups were determinated using Bonferroni's test

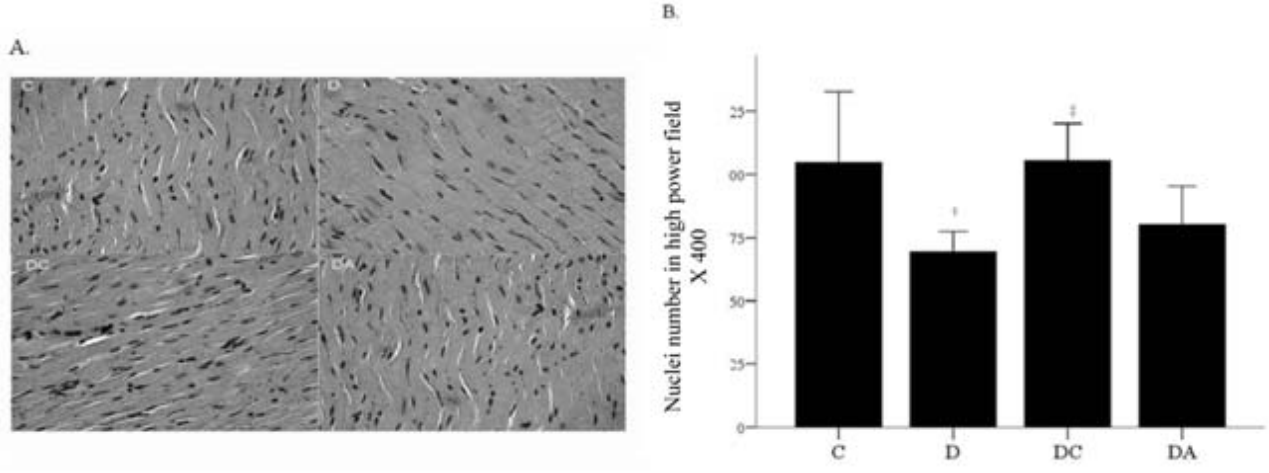

Figure 1. **A:** Hematoxylin and eosin staining for cardiac sections. Magnification × 400. **B:** Analysis of number of myocyte nuclei under high power field.
C = control, D = diabetic, DC = diabetic + captopril, DA = diabetic + aminoguanidine. †p <0.05 compared to the control group, ‡p <0.05 compared to the diabetic group. Pair-wise multiple comparisons between groups were determined using Bonferroni's test with $\alpha = 0.001$

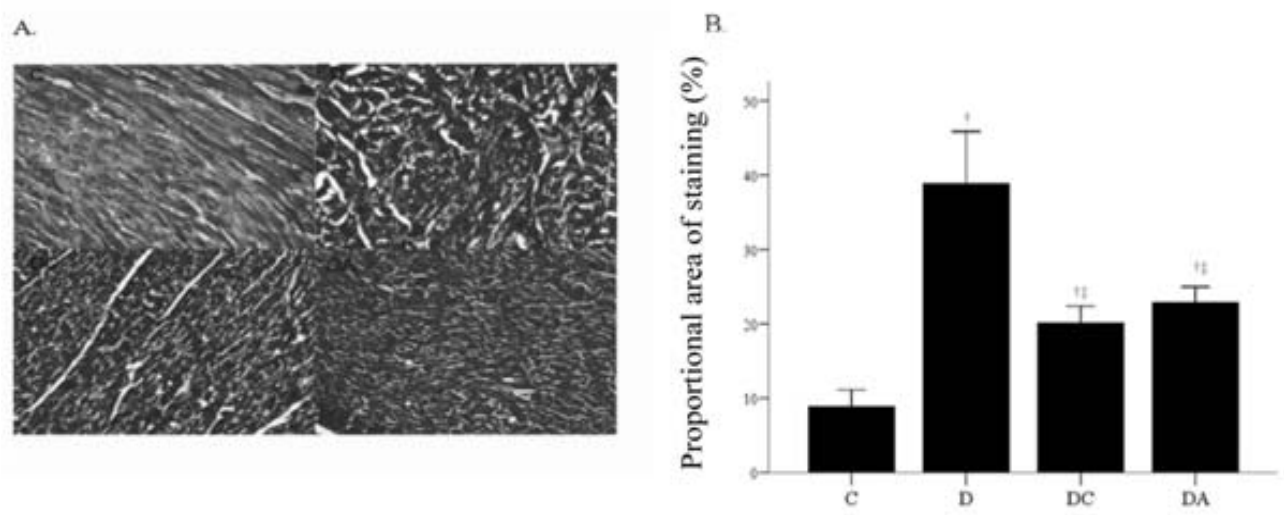

Figure 2. **A:** Masson trichrome staining for cardiac fibrosis. Magnification × 300. **B:** Morphometric analysis of staining for cardiac fibrosis.
C = control, D = diabetic, DC = diabetic + captopril, DA = diabetic + aminoguanidine. †p <0.05 compared to the control group, ‡p <0.05 compared to the diabetic group. Pair-wise multiple comparisons between groups were determined using Bonferroni's test with $\alpha = 0.001$

Accumulation of AGEs accumulation in diabetic myocardium

The accumulation of the AGEs biomarker CML and RAGEs was significantly higher in diabetic rats compared to non-diabetic control rats. This is shown in morphometric analysis (**Figure 3**) and Western blot analysis (**Figure 4**). Administration of captopril or aminoguanidine significantly decreased the magnitude of this accumulation. However, these two treatments showed only a trend to decrease the pentosidine accumulation seen in diabetic rats (**Figure 4A and B**).

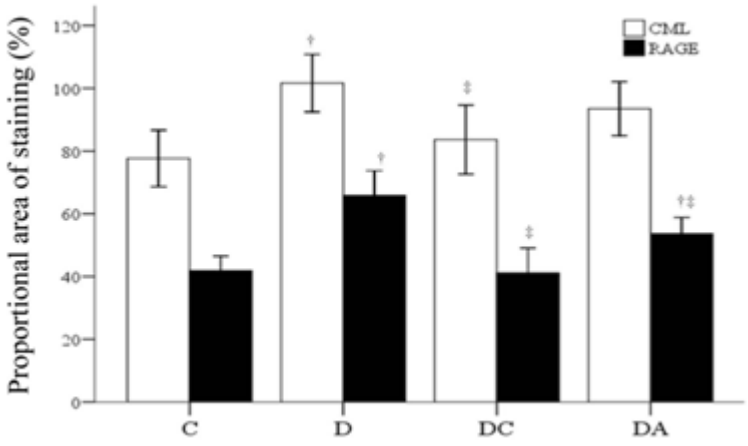

Figure 3. Morphometric analysis of immunostaining for cardiac CML and RAGE.
CML = carboxymethyllysine, C = control, D = diabetic, DC = diabetic + captopril, DA = diabetic + aminoguanidine. †p <0.05 compared to the control group, ‡p <0.05 compared to the diabetic group. Pair-wise multiple comparisons between groups were determined using Bonferroni's test with $\alpha = 0.001$

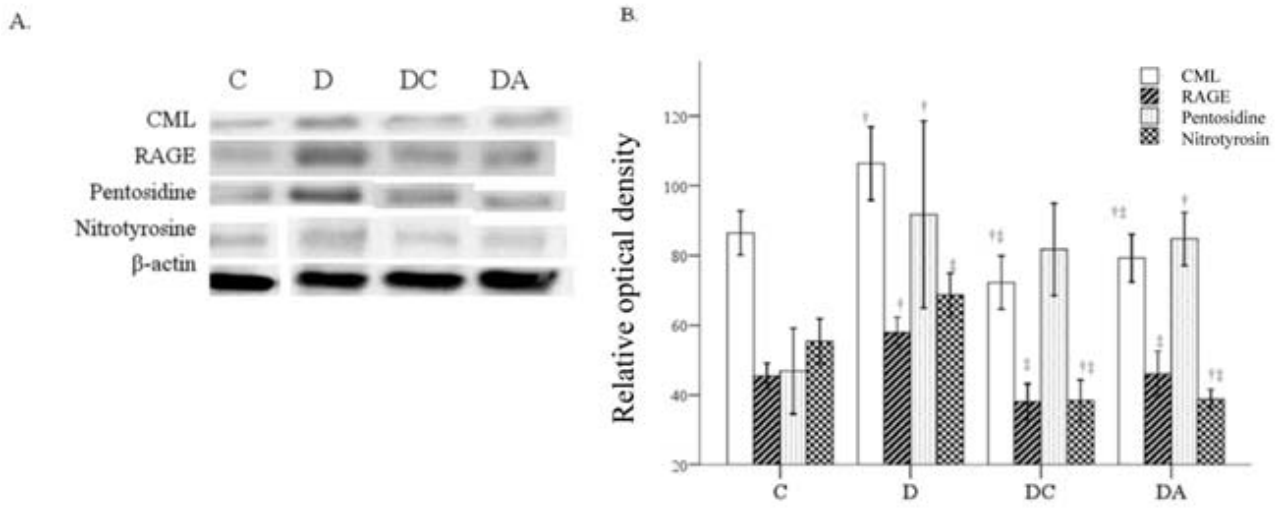

Figure 4. **A:** Representative Western blot analysis of cardiac CML, RAGE, pentosidine, and nitrotyrosine for all groups. β-actin as internal control. **B:** Quantitation of western blots for all groups.
C = control, D = diabetic, DC = diabetic + captopril, DA = diabetic + aminoguanidine. †p <0.05 compared to the control group, ‡p <0.05 compared to the diabetic group. Pair-wise multiple comparisons between groups were determined using Bonferroni's test with $\alpha = 0.001$

Oxidative stress in diabetic myocardium

Nitrotyrosine is an indicator of the production of the NO radical from oxidative stress, as well as an indicator of cell damage and inflammation. Myocardial nitrotyrosine levels were significantly elevated in diabetic rats, compared to controls. Both captopril and aminoguanidine treatment abolished the elevation.

Oxidative stress was also evaluated using an antibody for 8-OHdG, a biomarker for oxidative DNA damage induced by hydroxyl radicals. The number of 8-OHdG labeled nuclei, expressed as percentages of total myocyte nuclei in the myocardium, was significantly higher in diabetic myocardium than in that of control rats (2.6 1.3% vs. 0.5±0.2%, p <0.001). Captopril and aminoguanidine treatment abolished this diabetes-associated change (0.5±0. 1% vs. 2.6±1.3%, p <0.001 for both) as shown in **Figure 5**.

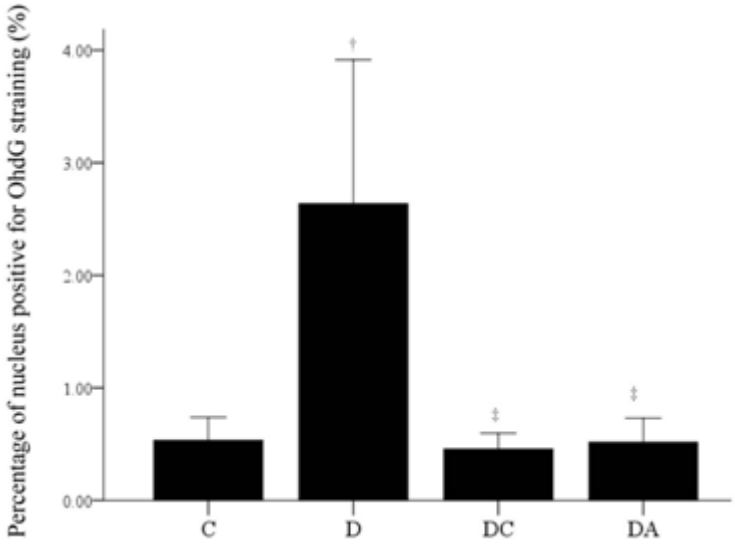

Figure 5. Proportion of myocyte nuclei positive for OHdG staining.
C = control, D = diabetic, DC = diabetic + captopril, DA = diabetic + aminoguanidine. †p <0.05 compared to the control group, ‡p <0.05 compared to the diabetic group. Pair-wise multiple comparisons between groups were determined using Bonferroni's test with $\alpha = 0.001$

Discussion

Hyperglycemia increases AGEs, angiotensin II and oxidative stress. In this study we investigated whether ACE inhibitors attenuated the elevations in AGEs and oxidative stress and whether this was an underlying mechanism for the alleviation of diabetic cardiomyopathy. We found that the ACE inhibitor captopril blocked augmentation of AGEs and oxidative stress to the same extent as aminoguanidine, an inhibitor of non-enzymatic glycation. In addition, ACE inhibition blocked the increment of collagen and BNP and the loss of myocytes seen in diabetic hearts. However, although it blocked the diabetes-caused increase in collagen to a similar degree as captopril, aminoguanosine only partially blocked the increase in BNP and had no effect on the myocyte loss.

The increased ROS production seen in our diabetic rats could be partially attributed to the elevated systolic blood pressure in the diabetic group. By stimulating the systemic and local RAS, both hyperglycemia and hypertension increase angiotensin II production [22]. Angiotensin II, in addition to causing vasoconstriction, stimulates the formation of ROS [12, 23, 24] and thereby leads to an oxidative stress response [25]. Therefore, inhibitory effects of captopril on the production in nitrotyrosine, the biomarker for oxidative stress, may result from the blockade of angiotensin production.

Hyperglycemia, in addition to stimulating the RAS, causes an increased formation of AGEs. Our observation that increased RAGE expression accompanied with elevated formation of AGEs agrees with a previous report showing that RAGE was induced in response to an increase in AGE [26]. AGEs have been shown to cross-link collagen, thus causing fibrosis [27]. Therefore, suppression of the diabetes-induced collagen increment by captopril is probably due to its inhibition of the diabetes-induced increase in AGEs. This reduction in AGEs accumulation caused by captopril has been attributed to inhibited generation of the free radicals that participate in the production of reactive carbonyls involved in the formation of AGEs [28]. In summary, captopril seems to attenuate formation of ROS, AGEs, and fibrosis by blocking the augmentation in ROS due to the increased angiotensin seen in diabetic cardiomyopathy.

Aminoguanidine decreases AGEs formation by scavenging dicarbonyl precursors to AGEs [11, 29] and reduces ROS production by inhibiting inducible nitric oxide synthase (NOS_i). Our data show that aminoguanidine resulted in a similar decrease in AGEs, ROS, and fibrosis to that caused by captopril. These data support the statement that captopril decreases the development of hyperglycemia-induced fibrosis probably via reduction of AGEs and ROS.

However, blockade of AGEs and ROS formation by aminoguanidine did not repeat all of the actions of captopril. Aminoguanidine did not block the hyperglycemia-induced loss of cardiomyocyte and only partially blocked the increase in BNP, the biomarker for cardiomyopathy, while captopril blocked both of these hyperglycemia-induced changes. This suggests that angiotensin has other actions in diabetic cardiomyopathy in addition to increasing AGEs and ROS. One possibility is its hypertensive effect. Angiotensin receptor blockers and ACE inhibitors, but not calcium channel blockers, inhibit the formation of AGEs in vitro [30], but this does not address the question of whether angiotensin-induced hypertension is responsible for non-AGE or ROS related effects. Growth factor-related actions of angiotensin might be another plausible explanation for the involvement of angiotensin in diabetic cardiomyopathy. This possibility needs further investigation.

Koka et al. [30] reported that AGEs activate chymase, which provides an alternate pathway for angiotensin generation. If this alternate pathway plays an important role in generating more angiotensin seen in diabetic cardiomyopathy, an angiotensin receptor blocker would be predicted to have a greater therapeutic effect than an ACE inhibitor. This possibility should be explored in the future. Another possibility to be explored is whether combined treatment with an ACE inhibitor and an AGEs formation inhibitor might have an additive or synergistic effect in diabetic cardiac dysfunction since Davis [31] has reported superior renal protective effects with combined ACE and AGE inhibition therapy in the diabetic spontaneously hypertensive rat.

Treatment with aminoguanidine or captopril led to a non-significant decrease in myocardial pentosidine levels in diabetic groups, although it significantly reduced the accumulation of CML, RAGE, and oxidative stress. A possible explanation is that the formation of this AGEs biomarker would requires oxidation as well as glycation [32], and therefore neither captopril nor aminoguanidine alone was able to attenuate its formation.

In addition, neither drug completely abolished the detrimental effects of hyperglycemia, which indicates

the presence of a reciprocal mechanism in the interaction between AGEs and RAS in diabetes. Aminoguanidine, primarily scavenges reactive dicarbonyl precursors to AGEs, has been found to block epidermal growth factor receptor signaling and consequent alterations in cellular homeostasis [33]. Captopril does not trap reactive carbonyl AGEs precursors such as methylglyoxal [11, 28], and its effects in the reducing AGEs accumulation have been attributed to inhibition of the generation of free radicals that participate in the production of reactive carbonyls [27]. In support of this hypothesis, we have shown in our study that captopril demonstrated antioxidant properties by its reduction of tissue nitrotyrosine levels. These observations all point to the mutual interactions between AGEs-mediated pathways and RAS-mediated oxidative stress in the development and progression of diabetic disease.

Our study employed a long-term STZ-induced type I diabetic model. This diabetic model may not be responsible for the most prevalent clinical type II diabetes. It was noteworthy that, in both treatment groups, we failed to observe the decrease of all cardiac AGEs in western blot expression. Perhaps a larger sample size or more analysis tools such as HPLC or GC will help to address this concern. AGEs, as post-translational modifications of peptides, are a complex mixture of trace compounds in tissue proteins, and their individual biological effects and precise mechanisms in increasing tissue damage as well as development of novel AGEs inhibitors are still under intensive investigation.

Conclusion

Our findings lead us to conclude that the anti-oxidant properties of ACE inhibitor are one of the underlying mechanisms in reducing diabetic cardiomyopathy, and may play a pivotal role in reducing the accumulation of AGEs and oxidative stress. The present study underlined the role of hyperglycemia-induced AGEs formation/accumulation and oxidative stress in diabetic cardiomyopathy, and the role of RAS in modulating these processes. In addition to the classical medical treatment of diabetes, a number of combined therapeutic approaches for neutralizing AGEs and reducing oxidative stress should clarify whether their implementation is of benefit in preventing diabetic complications.

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References

1. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. Am J Cardiol. 1974; 34:29-34.
2. Ren J, Davidoff AJ. Diabetes rapidly induces contractile dysfunctions in isolated ventricular myocytes. Am J Physiol. 1997; 272:H148-H158.
3. Poornima IG, Parikh P, Shannon RP. Diabetic cardiomyopathy: The search for a unifying hypothesis. Circ Res. 2006; 98:596-605.
4. Bell DS. Diabetic cardiomyopathy. A unique entity or a complication of coronary artery disease? Diabetes Care. 1995; 18:708-14.
5. Fu MX, Wells-Knecht KJ, Blackledge JA, Lyons TJ, Thorpe SR, Baynes JW. Glycation, glycoxidation, and cross-linking of collagen by glucose. Kinetics, mechanisms, and inhibition of late stages of the Maillard reaction. Diabetes. 1994; 43:676-83.
6. Ma H, Li SY, Xu P, Babcock SA, Dolence EK, Brownlee M, et al. Advanced glycation endproduct (AGE) accumulation and AGE receptor up-regulation contribute to the onset of diabetic nephropathy. J Cell Mol Med. 2009; 13:1751-64.
7. Edelstein D, Brownlee M. Aminoguanidine ameliorates albuminuria in diabetic hypertensive rats. Diabetologia. 1992; 35:96-7.
8. Hammes HP, Brownlee M, Edelstein D, Saleck M, Martin S, Federlin K. Aminoguanidine inhibits the development of accelerated diabetic retinopathy in the spontaneous hypertensive rat. Diabetologia. 1994; 37:32-5.
9. Cameron NE, Cotter MA, Dines K, Love A. Effects of aminoguanidine on peripheral nerve function and polyol pathway metabolites in streptozotocin-diabetic rats. Diabetologia. 1992; 35:946-50.
10. Fiordaliso F, Li B, Latini R, Sonnenblick EH, Anversa P, Leri A, et al. Myocyte death in streptozotocin-induced diabetes in rats in angiotensin II- dependent. Lab Invest. 2000; 80:513-27.
11. Fukagawa NK, Li M, Liang P, Russell JC, Sobel BE, Absher PM. Aging and high concentrations of glucose potentiate injury to mitochondrial DNA. Free Radical Biol Med. 1999; 27:1437-43.

12. Dunn JA, Patrick JS, Thorpe SR, Baynes JW. Oxidation of glycated proteins: age-dependent accumulation of N epsilon-(carboxymethyl)lysine in lens proteins. Biochemistry. 1989; 28:9464-8.
13. von Harsdorf R, Li PF, Dietz R. Signaling pathways in reactive oxygen species-induced cardiomyocyte apoptosis. Circulation. 1999; 99:2934-41.
14. Cooper ME. The role of the renin-angiotensin-aldosterone system in diabetes and its vascular complications. Am J Hypertension. 2004; 17:16S-20S; quiz A12-14.
15. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. Heart Outcomes Prevention Evaluation Study Investigators. Lancet. 2000; 355:253-9.
16. Ruiz-Ortega M, Lorenzo O, Ruperez M, Egido J. ACE inhibitors and AT(1) receptor antagonists-beyond the haemodynamic effect. Nephrol Dial Transplant. 2000; 15:561-5.
17. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N Engl J Med. 1993; 329:1456-62.
18. Nomoto T, Nishina T, Miwa S, Tsuneyoshi H, Maruyama I, Nishimura K, et al. Angiotensin-converting enzyme inhibitor helps prevent late remodeling after left ventricular aneurysm repair in rats. Circulation. 2002; 106:I115-9.
19. Wolf G, Neilson EG. Angiotensin II as a renal growth factor. J Am Soc Nephrol. 1993; 3:1531-40.
20. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem. 1981; 29:577-80.
21. Moreno PR, Murcia AM, Palacio IF, Leon MN, Bernardi VH, Fuster V, et al. Coronary composition and macrophage infiltration in atherectomy specimens from patients with diabetes mellitus. Circulation. 2000; 102:2180-4.
22. Westerman D, Rutschow S, Jager S, Linderer A, Anker S, Riad A, et al. Contributions of cardiac matrix metalloproteinase activity to cardiac failure in diabetic cardiuomyopathy.The role of angiotensin receptor antagonism. Diabetes. 2007; 56:641-6.
23. Lavrentyev EN, Estes AM, Malik KU. Mechanism of high glucose induced angiotensin II production in rat vascular smooth muscle cells. Circ Res. 2007; 101: 455-64.
24. Berry C, Hamilton CA, Brosnan MJ, Magill FG, Berg GA, McMurray JJ, et al. Investigation into the sources of superoxide in human blood vessels: angiotensin II increases superoxide production in human internal mammary arteries. Circulation. 2000; 101:2206-12.
25. Rueckschloss U, Quinn MT, Holtz J, Morawietz H. Dose-dependent regulation of NAD(P)H oxidase expression by angiotensin II in human endothelial cells: protective effect of angiotensin II type 1 receptor blockade in patients with coronary artery disease. Arterioscler Thromb Vasc Biol. 2002; 22:1845-51.
26. Tanaka N, Yonekura H, Yamagishi S, Fujimori H, Yamamoto Y, Yamamoto H. The receptor for advanced glycation end products is induced by the glycation products themselves and tumor necrosis factor-alpha through nuclear factor-kappa B, and by 17beta-estradiol through Sp-1 in human vascular endothelial cells. J Biol Chem. 2000; 275:25781-90.
27. Adeghate E. Molecular and cellular basis of the aetiology and management of diabetic cardiomyopathy: a short review. Mol Cell Biochem. 2004; 261:187-91.
28. Miyata T, van Ypersele de Strihou C, Ueda Y, Ichimori K, Inagi R, Onogi H, et al. Angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors lower in vitro the formation of advanced glycation end products: biochemical mechanisms. J Am Soc Nephrol. 2002; 13:2478-87.
29. Dunn JA, McCance DR, Thorpe SR, Lyons TJ, Baynes JW. Age-dependent accumulation of N epsilon-(carboxymethyl)lysine and N epsilon-(carboxymethyl)hydroxylysine in human skin collagen. Biochemistry. 1991; 30:1205-10.
30. Koka V. Wang W, Huang XR, Kim-Mitsuyama S, Truong LD, Lan HY. Advanced glycation end products activate a chymase-dependent angiotensin II-generating pathway in diabetic complication. Circulation. 2006; 113:1353-60
31. Davis BJ, Forbes JM, Thomas MC, Jerums G, Burns WC, Kawachi H, et al. Superior renoprotective effects of combination therapy with ACE and AGE inhibition in the diabetic spontaneously hypertensive rat. Diabetologia. 2004; 47:89-97
32. Grandhee SK, Monnier VM. Mechanism of formation of the Maillard protein cross-link pentosidine. Glucose, fructose, and ascorbate as pentosidine precursors. J Biol Chem. 1991; 266:11649-53.
33. Portero-Otin M, Pamplona R, Bellmunt MJ, Ruiz MC, Prat J, Salvayre R, et al. Advanced glycation end product precursors impair epidermal growth factor receptor signaling. Diabetes. 2002; 51:1535-42.