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COMMENTARY

Pharmacological prevention of cardiovascular aging – targeting the Maillard reaction

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The development of myocardial and large vessel stiffness with aging underlies the development of diastolic heart failure and isolated systolic hypertension. Nonenzymatic reaction between glucose and proteins (Maillard reaction) leading to collagen crosslinking in the myocardium and arterial wall has been implicated in age-related increase in cardiovascular stiffness. In the present issue, Chang *et al.* show that aminoguanidine, an inhibitor of protein crosslinking, retards age-related decline in the elastic properties of the left ventricle and arteries. The significance of these findings is discussed in this commentary.
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Abbreviations: AG, aminoguanidine

Introduction

Part of the normal aging process of humans and other long-lived species is the gradual decrease in the elasticity of the cardiovascular system, which leads to increased arterial and myocardial stiffness. The development of large vessel stiffness with aging underlies the development of isolated systolic hypertension. The prevalence of systolic hypertension increases steeply with age, especially after the sixth decade of life (Franklin *et al.*, 1997). A large body of evidence indicates that there is a strong link between stiffness of conduit vessels, systolic hypertension, and cardiovascular disease morbidity and mortality (Kannel, 1996; Aronson, 2003). In addition, arterial stiffness increases early wave reflection, leading to increased aortic and ventricular pressures during late systole and reduces aortic pressure during diastole. The resulting alteration in heart–vessel coupling increases left ventricular oxygen demands, predisposes to left ventricular hypertrophy and interstitial fibrosis, and slows left ventricular relaxation, thus contributing to the development of heart failure (Chen *et al.*, 1998; Haider *et al.*, 2003; Safar *et al.*, 2003).

A large proportion of older patients who present with symptoms of heart failure have normal left ventricular systolic function (also called 'diastolic heart failure'). The aging process depresses left ventricular diastolic performance, and heart failure due to diastolic dysfunction rises dramatically with age, leading to substantial morbidity and mortality (Banerjee *et al.*, 2004). There is increasing evidence that changes in the passive component of diastole (the component determined by the distensibility of the myocardium and is adversely affected by increased myocardial stiffness) and increased vascular stiffness account for the hemodynamic characteristics and symptoms of heart failure with normal ejection fraction (Chen *et al.*, 1998; Aronson, 2003; Kawaguchi *et al.*, 2003).

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Role of advanced glycation end products in the pathogenesis myocardial and vascular stiffness

One of the important mechanisms responsible for age-related increase in cardiovascular stiffness is the nonenzymatic reaction between glucose and proteins in arterial walls, collectively known as Maillard, or browning reaction (Cerami, 1985; Brownlee *et al.*, 1988; Ulrich & Cerami, 2001) (Figure 1). Maillard in 1912 incubated glucose with amino acids and observed the formation of yellow–brown pigments resulting from a nonenzymatic glycation reaction of glucose with protein. Glucose forms chemically reversible early glycation products with reactive amino groups of proteins (Schiff bases). Formation of the Schiff base from glucose and amine is relatively fast and highly reversible (Ulrich & Cerami, 2001), and represents an equilibrium reaction in which the amount of Schiff base formed is dictated by glucose concentration.

Over a period of days, the labile Schiff base subsequently rearranges to form the more stable Amadori-type early glycation products. Formation of Amadori product from the Schiff base is slower but much faster than the reverse reaction, and therefore tend to accumulate on proteins. Equilibrium levels of Amadori products are reached in weeks (Figure 1). As with the formation of the Schiff base, the amount of Amadori product formed is related to the glucose concentration (Ulrich & Cerami, 2001). The best-known Amadori product is hemoglobin A_{1C}, which is an adduct of glucose with the N-terminal valine amino group of the β-chain of hemoglobin.

Proteins bearing Amadori products are referred to as glycated proteins (distinguishing them from enzymatically

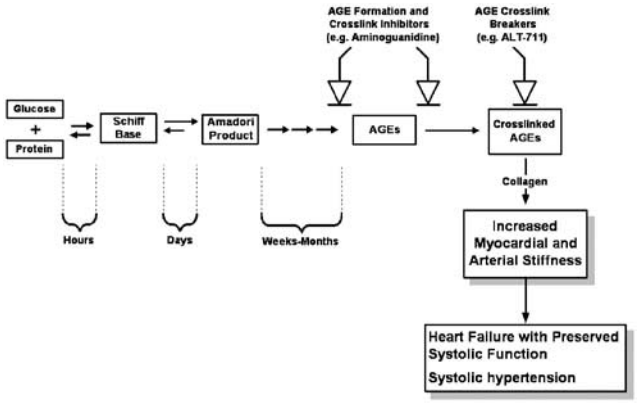

Figure 1 Schematic description of the formation of collagen crosslinks and strategies for blocking the formation of AGEs and AGE crosslinks. Glucose attaches to an amino group of a protein such as collagen to form a Schiff base, which subsequently transforms itself into an Amadori product. The latter can pass through several incompletely understood steps (broken arrows) to become an AGE. AGEs can react with free amino groups on an adjacent protein to form crosslinks. Crosslinking can lead to a decrease in large vessels and myocardial compliance, resulting in heart failure with preserved systolic function and/or systolic hypertension (see text for details).

glycosylated proteins), while the process of Amadori product formation is called glycation (Ulrich & Cerami, 2001). Some of the early glycation products on long-lived proteins (e.g. vessel wall collagen) continue to undergo complex series of chemical rearrangements *in vivo* to form complex compounds and crosslinks known as advanced glycation end products (AGEs) (Brownlee *et al*., 1988; Ulrich & Cerami, 2001) (Figure 1). An important characteristic of AGEs compared with the Amadori products is that, once formed, AGE-protein adducts are stable and virtually irreversible. The Maillard reaction proceeds normally in the body at homeostatic concentrations of glucose, resulting in AGEs accumulation with age, and at an accelerated rate in diabetes. However, only proteins with long half-lives such as collagen will accumulate substantial amounts of AGEs *in vivo* (Brownlee *et al*., 1988).

In the early 1980s, the potential importance of the complex, late-stage Maillard processes to age- and diabetes-related changes in the mechanical properties of vascular tissues has been recognized by Cerami (Monnier & Cerami, 1981; Cerami, 1985) (Figure 1).

The ability of AGE-modified proteins to form protein–protein crosslinks on collagen *in vivo* is a key determinant in the pathogenesis of the reduced vascular and myocardial compliance observed with aging and diabetes (Figure 1). The tensile strength and flexibility of collagen maintain the level of stiffness and elasticity necessary for normal functioning of the arteries. In contrast to post-translational covalent crosslinks within normal collagen, which occur only at two discrete sites at the N-terminal and C-terminal ends of the molecule, AGEs form crosslinks throughout the collagen molecule (Brownlee *et al*., 1986). In addition, one of the consequences of AGE crosslinking of collagen is decreased susceptibility to proteolytic and chemical degradation. The decreased proteolytic turnover results in an increased accumulation and continued AGE-derived crosslinking of myocardial and vessel wall collagen, leading to loss of elasticity and flexibility.

The pathophysiological significance of AGEs stems not only from their ability to modify the functional properties of proteins, but also from their interaction with cells *via* AGE binding proteins or AGE receptors. The cellular interactions of AGEs are mediated through a specific receptor for AGE determinants on cell surfaces (Schmidt *et al*., 1999). AGE interaction with cellular RAGE results in receptor activation and receptor-mediated induction of oxidative stress and inflammatory responses (Schmidt *et al*., 1999; 2001). Recent evidence suggests that activation of RAGE-dependent mechanisms may impair the active phase of diastolic relaxation. Exposure of cardiomyocytes to AGE caused a significant prolongation of Ca^{2+} transient decay, and this abnormality was aggravated by overexpression of RAGE. These results suggest that the AGE and RAGE could play an active role in the development of aging or diabetes-induced diastolic dysfunction (Petrova *et al*., 2002).

As elevated glucose concentrations accelerate glycation, collagen crosslinking occurs faster than expected by age alone in the presence of diabetes. Indeed, compared to nondiabetic individuals, patients with diabetes manifest premature development of both arterial stiffness (Lehmann *et al*., 1997) and diminished left ventricular compliance (Poirier *et al*., 2001; Aronson, 2003), leading to increased risk for congestive heart failure.

Pharmacologic modulation of AGE formation and AGE crosslinks in vascular tissue

Aminoguanidine (AG) is a prototype of scavenging agents that inhibit AGEs formation and protein-to-protein crosslinking (Brownlee *et al*., 1986). Prevention of AGE production and of protein crosslinking by AG was first described by Brownlee *et al*. (1986) *in vitro* and *in vivo* in diabetic rats. Subsequent studies showed that chronic administration of AG to Fischer 344 and Sprague–Dawley rats from 6 to 24 months resulted in a reduced content of AGEs in plasma, heart, blood vessels, and kidney (Li *et al*., 1996).

In this issue, Chang *et al*. (2004) investigate the effect of AG treatment on aged Fisher 344 rats. AG prevented the decline in effective arterial elastance in aging rats, suggesting that AG retards age-related decline in the elastic properties of the vasculature. In addition, AG prevented the reduction in left ventricular end-systolic elastance (normalized to left ventricular weight) that occurred in rats between 6 and 24 months of age. AG administration also increased the ratio of effective arterial elastance to left ventricular end-systolic elastance, which reflects the optimality of energy transmission from the left ventricle to the arterial system.

This study, together with previous studies in aging (Li *et al*., 1996; Corman *et al*., 1998) and diabetic animals (Huijberts *et al*., 1993; Norton *et al*., 1996), demonstrate the ability of AG to retard or prevent age- and diabetes-related deterioration in myocardial and arterial compliance, and underscore the important role of AGEs in the pathophysiology of age- and diabetes-related changes in the mechanical properties of vascular tissues. Unfortunately, the usefulness of AG in human studies was associated with significant side effects including gastrointestinal disturbances, abnormalities in liver function tests, flu-like symptoms, and vasculitis (Freedman

et al., 1999). Therefore, newer and less toxic compounds have been developed (e.g. pyridoxamine, ALT-946, OPB-9195) and are currently being used in clinical trials.

However, an important limitation of AG and other AGE formation inhibitors is that they cannot reverse pre-existing AGE crosslinking, and thus can be used only as preventive therapy and not for treatment of established disease. Recently, a new class of anti-AGE agents, containing a thiazolium structure that can break α-carbonyl compounds by cleaving the carbon–carbon bond between the carbonyls, has been developed (Vasan *et al*., 1996). Several studies have used the AGE 'breaker' compound 4,5-dimethyl-3-phenacylthiazolium chloride (DPTC, also called ALT-711 or Alagebrium) for animal and human studies (Ulrich & Cerami, 2001). In animals with pre-existing age- and diabetes-related vascular and myocardial stiffness, ALT-711 improved large vessel (Wolffenbuttel *et al*., 1998; Vaitkevicius *et al*., 2001) and myocardial (Asif *et al*., 2000) compliance. Preliminary studies have shown the efficacy of ALT-711 in humans (Kass *et al*., 2001). ALT-711 is presently in advanced phase II clinical trials for the treatment of systolic hypertension and heart failure with preserved systolic function.

Increasing understanding of the chemistry of the Maillard reaction led to the development of successful pharmacological interventions against AGE-induced crosslinking. Theoretically, the AGE crosslink breakers may have an advantage over inhibitors of AGE formation. The results of ongoing clinical studies will determine if these compounds can become the first specific therapy for age-related cardiovascular disorders.

References

ARONSON, D. (2003). Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. *J. Hypertens.,* **21,** 3–12.

ASIF, M., EGAN, J., VASAN, S., JYOTHIRMAYI, G.N., MASUREKAR, M.R., LOPEZ, S., WILLIAMS, C., TORRES, R.L., WAGLE, D., ULRICH, P., CERAMI, A., BRINES, M. & REGAN, T.J. (2000). An advanced glycation endproduct cross-link breaker can reverse age-related increases in myocardial stiffness. *Proc. Natl. Acad. Sci. U.S.A.,* **97,** 2809–2813.

BANERJEE, P., CLARK, A.L. & CLELAND, J.G. (2004). Diastolic heart failure: a difficult problem in the elderly. *Am. J. Geriatr. Cardiol.,* **13,** 16–21.

BROWNLEE, M., CERAMI, A. & VLASSARA, H. (1988). Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N. Engl. J. Med.,* **318,** 1315–1321.

BROWNLEE, M., VLASSARA, H., KOONEY, A., ULRICH, P. & CERAMI, A. (1986). Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science,* **232,** 1629–1632.

CERAMI, A. (1985). Hypothesis. Glucose as a mediator of aging. *J. Am. Geriatr. Soc.,* **33,** 626–634.

CHANG, K.-C., HSU, K.-L., CHOU, T.-F., LO, H.-M. & TSENG, Y.-Z. (2004). Aminoguanidine prevents age-related deterioration in left ventricular–arterial coupling in Fisher 344 rats. *Br. J. Pharmacol.*, **142,** 1099–1104.

CHEN, C.H., NAKAYAMA, M., NEVO, E., FETICS, B.J., MAUGHAN, W.L. & KASS, D.A. (1998). Coupled systolic-ventricular and vascular stiffening with age: implications for pressure regulation and cardiac reserve in the elderly. *J. Am. Coll. Cardiol.,* **32,** 1221–1227.

CORMAN, B., DURIEZ, M., POITEVIN, P., HEUDES, D., BRUNEVAL, P., TEDGUI, A. & LEVY, B.I. (1998). Aminoguanidine prevents age-related arterial stiffening and cardiac hypertrophy. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 1301–1306.

FRANKLIN, S.S., GUSTIN, W.T., WONG, N.D., LARSON, M.G., WEBER, M.A., KANNEL, W.B. & LEVY, D. (1997). Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation,* **96,** 308–315.

FREEDMAN, B.I., WUERTH, J.P., CARTWRIGHT, K., BAIN, R.P., DIPPE, S., HERSHON, K., MOORADIAN, A.D. & SPINOWITZ, B.S. (1999). Design and baseline characteristics for the aminoguanidine Clinical Trial in Overt Type 2 Diabetic Nephropathy (ACTION II). *Control. Clin. Trials,* **20,** 493–510.

HAIDER, A.W., LARSON, M.G., FRANKLIN, S.S. & LEVY, D. (2003). Systolic blood pressure, diastolic blood pressure, and pulse pressure as predictors of risk for congestive heart failure in the Framingham Heart Study. *Ann. Intern. Med.,* **138,** 10–16.

HUIJBERTS, M.S., WOLFFENBUTTEL, B.H., BOUDIER, H.A., CRIJNS, F.R., KRUSEMAN, A.C., POITEVIN, P. & LEVY, B.I. (1993). Aminoguanidine treatment increases elasticity and decreases fluid filtration of large arteries from diabetic rats. *J. Clin. Invest.,* **92,** 1407–1411.

KANNEL, W.B. (1996). Blood pressure as a cardiovascular risk factor: prevention and treatment. *JAMA,* **275,** 1571–1576.

KASS, D.A., SHAPIRO, E.P., KAWAGUCHI, M., CAPRIOTTI, A.R., SCUTERI, A., DEGROOF, R.C. & LAKATTA, E.G. (2001). Improved arterial compliance by a novel advanced glycation end-product crosslink breaker. *Circulation,* **104,** 1464–1470.

KAWAGUCHI, M., HAY, I., FETICS, B. & KASS, D.A. (2003). Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations. *Circulation,* **107,** 714–720.

LEHMANN, E.D., RILEY, W.A., CLARKSON, P. & GOSLING, R.G. (1997). Non-invasive assessment of cardiovascular disease in diabetes mellitus. *Lancet,* **350,** SI14–SI19.

LI, Y.M., STEFFES, M., DONNELLY, T., LIU, C., FUH, H., BASGEN, J., BUCALA, R. & VLASSARA, H. (1996). Prevention of cardiovascular and renal pathology of aging by the advanced glycation inhibitor aminoguanidine. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 3902–3907.

MONNIER, V.M. & CERAMI, A. (1981). Nonenzymatic browning *in vivo*: possible process for aging of long-lived proteins. *Science,* **211,** 491–493.

NORTON, G.R., CANDY, G. & WOODIWISS, A.J. (1996). Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats. *Circulation,* **93,** 1905–1912.

PETROVA, R., YAMAMOTO, Y., MURAKI, K., YONEKURA, H., SAKURAI, S., WATANABE, T., LI, H., TAKEUCHI, M., MAKITA, Z., KATO, I., TAKASAWA, S., OKAMOTO, H., IMAIZUMI, Y. & YAMAMOTO, H. (2002). Advanced glycation endproduct-induced calcium handling impairment in mouse cardiac myocytes. *J. Mol. Cell. Cardiol.,* **34,** 1425–1431.

POIRIER, P., BOGATY, P., GARNEAU, C., MAROIS, L. & DUMESNIL, J.G. (2001). Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. *Diabetes Care,* **24,** 5–10.

SAFAR, M.E., LEVY, B.I. & STRUIJKER-BOUDIER, H. (2003). Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation,* **107,** 2864–2869.

SCHMIDT, A.M., YAN, S.D., WAUTIER, J.L. & STERN, D. (1999). Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ. Res.,* **84,** 489–497.

SCHMIDT, A.M., YAN, S.D., YAN, S.F. & STERN, D.M. (2001). The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J. Clin. Invest.,* **108,** 949–955.

ULRICH, P. & CERAMI, A. (2001). Protein glycation, diabetes, and aging. *Recent Prog. Horm. Res.,* **56,** 1–21.

VAITKEVICIUS, P.V., LANE, M., SPURGEON, H., INGRAM, D.K., ROTH, G.S., EGAN, J.J., VASAN, S., WAGLE, D.R., ULRICH, P., BRINES, M., WUERTH, J.P., CERAMI, A. & LAKATTA, E.G. (2001). A cross-link breaker has sustained effects on arterial and ventricular properties in older rhesus monkeys. *Proc. Natl. Acad. Sci. U.S.A.,* **98,** 1171–1175.

VASAN, S., ZHANG, X., ZHANG, X., KAPURNIOTU, A., BERNHAGEN, J., TEICHBERG, S., BASGEN, J., WAGLE, D., SHIH, D., TERLECKY, I., BUCALA, R., CERAMI, A., EGAN, J. & ULRICH, P. (1996). An agent cleaving glucose-derived protein crosslinks *in vitro* and *in vivo* (see comments). *Nature,* **382,** 275–278.

WOLFFENBUTTEL, B.H., BOULANGER, C.M., CRIJNS, F.R., HUIJBERTS, M.S., POITEVIN, P., SWENNEN, G.N., VASAN, S., EGAN, J.J., ULRICH, P., CERAMI, A. & LEVY, B.I. (1998). Breakers of advanced glycation end products restore large artery properties in experimental diabetes. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 4630–4634.

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