

Cross-link breakers as a new therapeutic approach to cardiovascular disease

D. Susic¹

Hypertension Research Laboratory, Division of Research, Ochsner Clinic Foundation, 1520 Jefferson Highway, New Orleans, LA 70121, U.S.A.

Abstract

Fibrillar proteins, such as collagens type I and III, and elastin are components of the extracellular matrix. They form an intricate widespread network that provides a basis for maintaining the physical structure of the heart and vessels and also play an important role in determining cardiovascular function. Physiologically, collagen and elastin fibres are enzymatically cross-linked to form matrix. In addition to these enzymatically formed cross-links, collagen fibres may be linked non-enzymatically, most notably by formation of AGEs (advanced glycation end-products). AGEs are formed by a reaction between reducing sugars and body proteins; they are formed increasingly in diabetes mellitus and hypertension and they accumulate with aging. There are several mechanisms whereby AGEs may affect cardiovascular structure and function. These include increased myocardial and vascular stiffness and (upon reaction with their receptors) inflammatory reactions, release of growth factors and cytokines, and increased oxidative stress. Therefore breaking AGEs appears as a promising tool in the therapy of cardiovascular injury related to diabetes, hypertension and aging. Breakers of AGE cross-links have been developed and one of them, alagebrium, has been extensively studied. This brief review discusses the formation of AGEs, their role in mediating cardiovascular injury, as well as the results of experimental and clinical studies involving alagebrium.

The unique organization of collagen and elastin fibres in the extracellular matrix of cardiovascular tissue provides the basis for its structural integrity and optimal function. In myocardium, collagen fibres are enzymatically cross-linked to form collagen matrix, which in turn provides support for myocytes, thus ensuring normal function [1]. Similarly, in vascular tissue, enzymatically formed cross-links in collagen and elastin are essential for the mechanical stability of respective fibres, which provides strength and elasticity to vessels. It has also been postulated that alterations in collagen may contribute significantly to the impairment of cardiovascular function [2–4]. Thus changes in the absolute amount of collagen or collagen types have been associated with altered function of myocardium and vessels [2–6]. In addition, other variables (such as extensive cross-linking of collagen) due to glycation of proteins and formation of AGEs (advanced glycation end-products) may affect the structure and function of fibrillar collagen and extracellular matrix. AGEs are formed by a reaction between reducing sugars and body proteins [7]; they slowly accumulate during the life span, and their formation is accelerated in diabetes mellitus and hypertension. Importantly, AGEs adversely affect cardiovascular structure and function. Increased collagen cross-linking increases vascular and myocardial stiffness; this leads to elevated systolic and pulse pressures, impaired ventricular

relaxation and diastolic dysfunction. Furthermore, AGEs interact with receptors on endothelial- and smooth-muscle cells with consequent inflammatory reactions, release of growth factors and cytokines and increased oxidative stress.

Formation of AGEs

The formation of AGEs is a complex and lengthy process involving several pathways. Reducing sugars, such as glucose and ribose, have an aldehyde group at one end that bonds to an amino group of protein, usually at lysine and arginine residue [7]. This covalent reaction is called glycation. The first product in the formation of AGEs is Schiff base, then Amadori products of which the best known is haemoglobin A1C. Over time, glycation adduct on protein chain covalently bonds to a second protein chain, forming a permanent cross-link. Three different molecular structures are encountered in all glycation cross-links, and they are, in the order of abundance: (i) glucosepane, (ii) α -diketone and (iii) lysine-dihydropyridinium-lysine [7–9]. Once formed, the AGEs are highly resistant to proteolytic degradation [10]; and they apparently inhibit activation of matrix metalloproteinase-2, thereby promoting collagen accumulation and fibrosis [10].

Functional significance of AGEs

Increased formation of AGEs occurs naturally with aging and is greatly enhanced by hyperglycaemia and hypertension [11–13]. Consequently, the untoward effects of AGEs may be expected in the elderly and in patients with diabetes mellitus or hypertension. There are several mechanisms whereby AGEs may affect cardiovascular structure and function [12–15].

Key words: advanced glycation end-product (AGE), alagebrium, cardiovascular injury, collagen fibre, cross-link breaker, glycation.

Abbreviations used: ACE, angiotensin-converting enzyme; AGE, advanced glycation end-product; PWV, pulse wave velocity; RAGE, receptor for advanced glycation end-product; SHR, spontaneously hypertensive rat.

¹email dsusic@ochsner.org

Perhaps the most obvious and most studied is related to increased protein cross-linking and altered cardiovascular stiffness. Since formation of AGEs requires considerable time, they can form only on long-lived proteins such as collagen. As collagen fibres are the main components of extracellular matrix providing structural integrity to cardiovascular tissue, it is obvious that increased collagen cross-linking can affect vascular and myocardial stiffness. Increased collagen and elastin cross-linking in aorta and large arteries decreases distensibility, thereby increasing systolic and pulse pressure, pulse wave velocity, and late augmentation of systolic pressure. In turn, these changes impose an additional burden to the left ventricle contributing to the development of hypertrophy, heart failure and myocardial ischaemia. Increased myocardial stiffening results in impairments of ventricular relaxation and diastolic dysfunction. Another mechanism whereby AGEs may induce cardiovascular injury is through interaction with their receptors [RAGEs (receptors for advanced glycation end-product)] located on endothelial- and smooth-muscle cells and on myocytes [12–15]. These interactions may result in inflammatory reactions, release of growth factors and cytokines and increased oxidative stress. In cardiac myocytes, interaction with AGEs may affect calcium handling, thus affecting ventricular relaxation [16]. Finally, AGEs may affect low-density lipoprotein handling, thus contributing to atherogenesis [17].

In view of the foregoing, interference with AGEs appears to be a promising tool in the prevention and therapy of cardiovascular injury related to hyperglycaemia, aging and hypertension. There are several possible approaches including: (i) inhibition of AGEs formation, (ii) blocking AGE receptors and (iii) breaking AGE-related cross-links. Several agents, including aminoguanidine and OPB 9195, that inhibit AGEs formation have been shown to be effective in treating cardiovascular injury [18,19]. Interestingly, ACE (angiotensin-converting enzyme) inhibitors and angiotensin receptor blockers have been also shown to inhibit AGE formation *in vitro* [20]. Furthermore, few studies with soluble RAGE and RAGE antibodies indicated that blocking AGE receptors may have beneficial effects after vascular injury [14,21]. Further discussion will be limited to AGE-related protein cross-links breakers.

Breakers of AGE-related protein cross-links: studies with alagebrium

Experimental studies

Further advances on the role of protein glycation in the pathogenesis of disorders accompanying aging, diabetes and hypertension came with the discovery of breakers of AGE-related cross-links [22]. One of them, alagebrium (ALT-711; 4,5-dimethyl-3-phenacylthiozolium chloride) that acts only on α -diketone cross-links has been almost exclusively studied. One of the first studies examined the effects of alagebrium on mechanical properties of carotid arteries in diabetic rats [23]. The results demonstrated that alagebrium

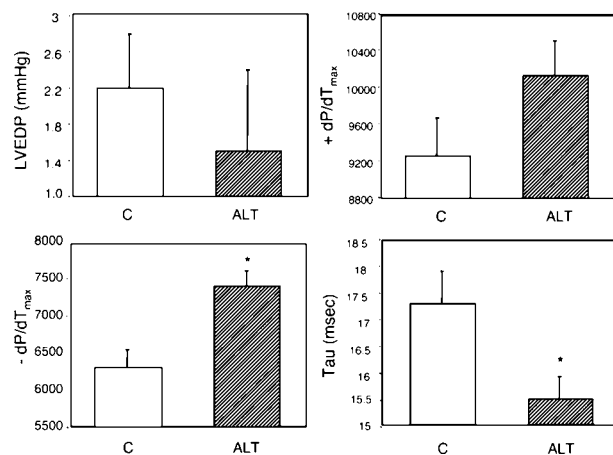
significantly improved carotid artery cross-sectional compliance [23]. This was the first *in vivo* study to demonstrate that breaking already formed AGE cross-links may ameliorate the adverse cardiovascular effects of diabetes. Subsequent studies in aged dogs and monkeys extended these observations. In aged dogs, alagebrium enhanced cardiac diastolic compliance, which was associated with improved diastolic filling and cardiac output [24]. Studies in older monkeys demonstrated that alagebrium improved both arterial and ventricular function, as evidenced by decreased pulse wave velocity and augmentation index of carotid pressure and improved stroke volume index and fractional shortening [25]. One study examined the combined effects of aging and diabetes on left ventricular function, myocardial collagen content and aortic distensibility as well as whether alagebrium may improve function of the heart and reduce collagen content [26]. That study was performed in 9–12-year-old mongrel dogs with aloxan-induced diabetes. Animals were studied before diabetes and 5 months after the induction of diabetes. At that point, one-half of the diabetic dogs received alagebrium, the other half received placebo, and they were studied 1 month after the initiation of respective treatments. Induction of diabetes in aging dogs increased heart mass by 14%, decreased left ventricular function, as shown by a fall in the ejection fraction, increased myocardial collagen content (both types I and III), and increased aortic stiffness. Treatment with alagebrium prevented the diabetes-induced increase in heart mass, restored ejection fraction and reduced aortic stiffness. Furthermore, the treatment also increased left ventricular collagen solubility and reduced collagen content. These results clearly indicate that AGE-related collagen cross-linking has a causative role in the development of cardiovascular complications of diabetes and aging. Two studies in diabetic rats also demonstrated that, in addition to reducing vascular and myocardial stiffness by breaking collagen cross-links, beneficial cardiovascular effects of alagebrium may be mediated by reduction of profibrotic growth factors and cytokines, as well as by diminishing oxidative stress [27,28].

We examined the cardiovascular and renal effects of alagebrium in adult and aging SHR (spontaneously hypertensive rats) [29]. That study included several experiments performed in 40–80-week-old male SHR. In all experiments, alagebrium insignificantly lowered arterial pressure except one experiment in which 60-week-old rats were given either placebo or alagebrium for two months, and systolic pressure (tail-cuff) was determined at weekly intervals. In the treated rats, arterial pressure decreased from 203 ± 3 to 187 ± 3 mmHg ($P < 0.05$) (paired *t* test) but no change was noted in the control group (from 205 ± 4 to 201 ± 3 mmHg). Left ventricular and aortic mass indices were consistently reduced by ALT-711 in all treated rats.

More recently, we examined the cardiovascular and renal effects of prolonged treatment (over 6 months) with alagebrium in 10-month-old SHR (D. Susic, J. Varagic and E.D. Froheich, unpublished work). To this end, initial determinations of 24 h urinary protein excretion and systolic arterial pressure (tail-cuff) were made in 100 SHR. These

Figure 1 | Left ventricular function in control (c) and alagebrium-treated (ALT) SHR

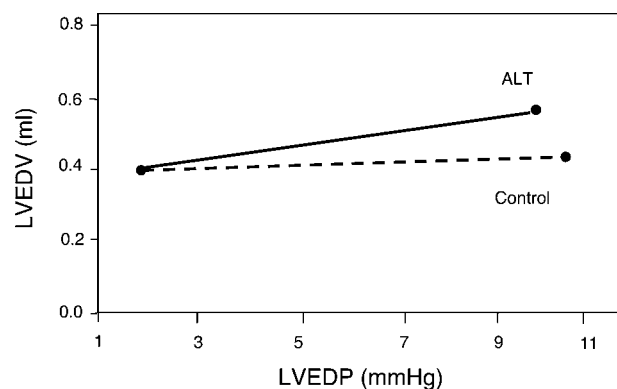
LVEDP, left ventricular end-diastolic pressure; $+dP/dT_{\max}$, maximal rate of pressure rise; $-dP/dT_{\max}$, maximal rate of pressure decline; tau, diastolic time constant. * $P < 0.05$ when compared with control.



rats were then divided into two groups, 50 in each, that were closely matched as to baseline urinary protein excretion and arterial pressure. At that point, one group received alagebrium ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$), whereas the other received vehicle (distilled water) by gastric gavage. Determinations of urinary protein excretion were repeated 3 and 6 months later in all rats. When the SHRs were approx. 70 weeks old, 15 rats from each group were randomly selected for haemodynamic study. The remaining rats continued to receive their respective treatment until death and they were used for survival study. The haemodynamic studies were performed in anaesthetized rats. A catheter-tip transducer was placed into the left ventricle via right carotid artery; the other was placed into descending aorta (close to bifurcation), via femoral artery. After measurement of several indices of cardiac function, left ventricular catheter was pulled out of the ventricle into ascending aorta. PWV (pulse wave velocity) and aortic distensibility (aortic diameter measured by echocardiography simultaneously with pressure recordings) were then measured. Urinary protein excretion increased with time (34.8 ± 1.7 , 58.5 ± 2.3 and $95.5 \pm 2.6 \text{ mg/24 h}$, at baseline and 3 and 6 months later respectively) and this increase was significantly attenuated in alagebrium-treated rats (36.1 ± 1.6 , 48.6 ± 2.0 and $68.2 \pm 2.4 \text{ mg/24 h}$). Haemodynamic studies revealed no difference in arterial pressure, cardiac index, total peripheral resistance and heart rate between the groups. Aortic distensibility was significantly greater in alagebrium-treated rats than in controls (1.12 ± 0.08 versus $0.73 \pm 0.07 \mu\text{m/mmHg}$, $P < 0.05$), whereas PWV was reduced in treated rats (435 ± 13 versus $499 \pm 12 \text{ cm/s}$, $P < 0.05$); both findings suggesting reduced aortic stiffness in rats receiving alagebrium. No difference in the left ventricular end-diastolic pressure and maximal rate of pressure rise between the groups was found (Figure 1). Two indices of left ventricular diastolic function, maximal rate of pressure fall and diastolic time

Figure 2 | Left ventricular pressure minus volume ratio determined before and after volume load (3 ml/100 g of body weight of 4% albumin solution)

LVEDV, left ventricular end-diastolic volume; LVEDP, left ventricular end-diastolic pressure.



constant, improved in treated rats (Figure 1). Left ventricular pressure/volume ratio determined before and after volume load (3 ml/100 g of body weight of 4% albumin solution) indicated somewhat decreased left ventricular stiffness in treated rats (Figure 2). Life span was also extended in alagebrium-treated rats. Control rats lived 84 ± 2 weeks, whereas treated rats lived 92 ± 2 ($P < 0.05$). Approximately 50% of placebo-treated rats died with signs of left ventricular failure (dyspnoea, pulmonary oedema and extremely dilated hearts); another 20% had tumours; approx. 10% showed signs of stroke; cause of death was not obvious in the remaining rats. Interestingly, treatment with alagebrium did not alter these percentages; the rats just died older.

Clinical studies

The cardiovascular effects of alagebrium have also been examined in a few clinical studies [30–32]. In older human subjects with elevation of arterial pulse pressure and reduced arterial compliance at the initiation of the study, the 8-week treatment with alagebrium lowered pulse pressure and improved arterial compliance [30]. Initial results in trials in patients with systolic hypertension and mean age of 66–68 years demonstrated no significant effect of several different doses of alagebrium [31]. However, post hoc analysis demonstrated an effect on systolic pressure in several dose groups, particularly in more severely affected patients [31]. Finally, the efficacy of alagebrium was also tested in patients with diastolic heart failure [32]. Exercise tolerance, aortic distensibility, left ventricular hypertrophy, diastolic filling and quality of life were assessed in 23 patients with diastolic heart failure. Treatment for 16 weeks with alagebrium reduced left ventricular mass, improved left ventricular diastolic filling and had a positive effect on quality of life measurements. However, exercise tolerance, arterial pressure and aortic distensibility remained unchanged.

In summary, the results of clinical trials so far have shown some beneficial cardiovascular effects of alagebrium

in elderly patients with increased systolic pressure, diastolic heart failure or increased aortic stiffness. However, the effects were far less than could be expected from the results of animal studies. It should be noted that alagebrium breaks only one type of cross-links (α -diketone) but does not affect the more abundant glucosepane cross-links. Furthermore, majority of patients in these trials were treated for co-morbid events with various drugs including angiotensin II receptor blockers and ACE inhibitors, which are known to inhibit AGE formation and may, therefore, mask the effect of alagebrium. Additional clinical studies are certainly needed to determine utility of alagebrium in treating cardiovascular disorders.

Conclusion

Formation of AGEs and its pathophysiological consequences contribute to the cardiovascular disorders associated with aging, diabetes and hypertension. Pharmacological approaches aimed at abolishing these adverse actions involve agents that can either prevent AGEs formation, break existing cross-links, or block receptors. Numerous reports clearly indicate that breaking AGE-related protein cross-links may exert beneficial cardiovascular effects by reducing vascular and myocardial stiffness, decreasing levels of growth factors and cytokines, and by diminishing oxidative stress.

References

- Jugdutt, B.I. (2003) *Curr. Drug Targets Cardiovasc. Haematol. Disord.* **3**, 1–30
- Weber, K.T., Sun, Y. and Guarda, E. (1994) *Hypertension* **23**, 869–877
- Covell, J.W. (1990) *Circulation* **81** (Suppl. III), 155–158
- Diez, J., Gonzales, A., Lopez, B. and Querjeta, R. (2005) *Nat. Clin. Pract. Cardiovasc. Med.* **2**, 209–216
- Spinale, F.G., Coker, M.L., Bond, B.R. and Zellner, J.L. (2005) *Cardiovasc. Res.* **46**, 225–238
- Zile, M.R., Baicu, C.F. and Gaasch, W.H. (2004) *N. Engl. J. Med.* **350**, 1953–1959
- Bucala, R. and Cerami, A. (1992) *Adv. Pharmacol.* **23**, 1–34
- Sell, D.R., Biernell, K.M., Reihl, O., Lederer, M.O., Strauch, C.M. and Monnier, V.M. (2005) *J. Biol. Chem.* **280**, 12310–12315
- Cheng, R., Feng, Q., Argirov, O.K. and Orthwert, B.J. (2004) *J. Biol. Chem.* **279**, 45441–45449
- Kuzuya, M., Asai, T., Kanda, S., Maeda, K., Cheng, X.W. and Iguchi, A. (2001) *Diabetologia* **44**, 433–436
- Bucala, R. and Cerami, A. (1992) *Adv. Pharmacol.* **23**, 1–34
- Lee, A.T. and Cerami, A. (1992) *Ann. N.Y. Acad. Sci.* **663**, 63–70
- Aronson, D. (2003) *J. Hypertens.* **21**, 3–12
- Zieman, S.J. and Kass, D.A. (2004) *Drugs* **64**, 459–470
- Susic, D., Varagic, J., Ahn, J. and Frohlich, E.D. (2004) *Curr. Opin. Cardiol.* **19**, 336–340
- Petrova, R., Yamamoto, Y., Muraki, K., Yonekura, H., Sakurai, S., Watanabe, T., Li, O.H., Takeuchi, M., Makita, Z., Kato, I. et al. (2002) *J. Mol. Cell. Cardiol.* **34**, 1425–1431
- Bucala, R., Mitchell, R., Arnold, K., Innerarity, T., Vlassara, H. and Cerami, A. (1995) *J. Biol. Chem.* **270**, 10828–10832
- Corman, B., Duriez, M. and Poitevin, P. (1998) *Proc. Natl. Acad. Sci. U.S.A.* **95**, 1301–1306
- Mizutani, K., Ikeda, K., Tsudi, K. and Yamori, Y.J. (2002) *Hypertension* **20**, 1607–1614
- Miyata, T. and van Ypersele de Strihou, C. (2003) *Arch. Biochem. Biophys.* **419**, 50–54
- Simm, A., Bartling, B. and Silber, R.E. (2004) *Ann. N.Y. Acad. Sci.* **1019**, 228–231
- Vasan, S., Zhang, X., Zhang, S., Kapurniotou, A., Bernhagen, J., Teichber, S., Basgen, J., Wagle, D., Shih, D. and Terlecky, I. (1996) *Nature* **382**, 275–278
- Wolfenbutell, B.H.R., Boulanger, C.M., Crijns, F.R.L., Huijberts, M.S.P., Poievin, P. and Swennen, G.N.M. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **95**, 4630–4634
- Asif, M., Egan, J., Vasan, S., Jyothirmayi, G.N., Masurekar, M.R., Lopey, S., Williams, C., Torres, R.L., Wagle, D., Ulrich, P. and Cerami, A. (2000) *Proc. Natl. Acad. Sci. U.S.A.* **97**, 2809–2813
- Vaitkevicius, P.V., Lane, M., Spurgeon, H., Ingram, D.K., Roth, G., Egan, J.J., Vasan, S., Wagle, D.R., Ulrich, P., Brines, M. et al. (2001) *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1171–1175
- Liu, J., Masurekar, M.R., Vatner, D.E., Jyothirmayi, G.N., Regan, T.J., Vatner, S.F., Meggs, L.G. and Malhotra, A. (2003) *Am. J. Physiol. Heart Circ. Physiol.* **285**, H2587–H2591
- Candido, R., Forbes, J.M., Thomas, M.C., Thallas, V., Dean, R.G., Burns, W.C., Tikellis, C., Ritchie, R.H., Twigg, S.M., Cooper, M.E. and Burrell, L.M. (2003) *Circ. Res.* **92**, 785–792
- Forbes, J.M., Thallas, V., Thomas, M.C., Founds, H.W., Burns, W.C., Jerums, G. and Cooper, M.E. (2003) *FASEB J.* **17**, 1762–1764
- Susic, D., Varagic, J., Ahn, J. and Frohlich, E.D. (2004) *Am. J. Hypertens.* **17**, 328–333
- Kass, D.A., Shapiro, E.P., Kawaguchi, M., Capriotti, A.R., Scuteri, A., DeGroof, R. and Lakata, E.G. (2001) *Circulation* **104**, 1464–1470
- Bakris, G.L., Bank, A.J., Kass, D.A., Neutel, J.M., Preston, R.A. and Oparil, S. (2004) *Am. J. Hypertens.* **17**, 205–205
- Little, W.C., Zile, M.R., Kitzman, D.W., Hundley, W.G., O'Brien, T.X. and deGroof, R.C. (2005) *J. Card. Failure* **11**, 191–195

Received 11 June 2007
doi:10.1042/BST0350853