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The Accumulation of the Glycoxidation Product N^{ε}-carboxymethyllysine in Cardiac Tissues with Age, Diabetes Mellitus and Coronary Heart Disease

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Heart disease is one of the most important causes of death in developed countries. N^{ε}-carboxymethyllysine (CML) is a major advanced glycation end product formed by combined reactions of non-enzymatic glycation and oxidation (glycoxidation), and it represents a general marker of oxidative stress. CML has been suggested to be involved in the pathogenesis of heart disease. Plasma CML is elevated in aging, atherosclerosis and/or diabetes. In this study, we measured cardiac CML levels to elucidate its role in the pathogenesis of heart disease. Cardiac tissues were collected from 105 patients (55.6 ± 17.0 years old: age range, 1-78 years) undergoing cardiac surgery. The diseases comprised coronary heart disease (CHD), CHD associated with diabetes mellitus (DM), valvular heart disease and congenital heart disease. The concentration of CML in cardiac tissues of each group was 4.31 ± 0.66, 5.29 ± 0.59, 2.74 ± 1.05 and 1.75 ± 1.16 μg/g, respectively. ELISA was used for measuring cardiac and plasma CML concentrations. Multiple linear regression analysis showed a significant positive correlation of CML concentrations with age (r = 0.803, p < 0.001), DM (r = 0.567, p < 0.001) and CHD (r = 0.523 p < 0.001). R^2 was 0.872 (p < 0.001); the three independent variables could explain 87.2% variation of CML concentrations. Cardiac CML concentrations exhibited a significant positive correlation with plasma CML (r = 0.983, p < 0.001). Our data indicate that cardiac CML concentrations increase with age, DM and/or CHD, and exhibit a positive correlation with plasma CML concentrations.

Keywords: advanced glycation end product; age; coronary heart disease; diabetes mellitus; N^{ε}-carboxymethyllysine
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Introduction

Heart disease is one of the most important causes of death in developed countries, and it predominantly comprises coronary heart disease (CHD) and heart failure (HF). The morbidity from heart disease increases with age, and diabetes mellitus (DM) is associated with an increased risk of heart disease after adjustment for age (Day et al. 1988; Stanislawska et al. 1993; Mak et al. 1997; Fujiwara et al. 2002; Bauters et al. 2003; Hirakawa et al. 2007; Jensen et al. 2012). Poor glycemic control is independently associated with the incidence of hospitalization and/or death due to CHD and HF among adult patients with DM (Day et al. 1988; Stratton et al. 2000; Iribarren et al. 2001; Fujiwara et al. 2002; Bauters et al. 2003; Hirakawa et al. 2007). A variety of mechanisms have been proposed, and it is currently thought that the increased risk of CHD and HF with hyperglycemia may be causally related to both micro- and macroangiopathy (Stratton et al. 2000; Brener et al. 2012). An important role in the acceleration of vascular disease has been previously suggested for advanced glycation end (AGE) products (AGEs) (Lieuw-a-Fa et al. 2006). AGEs are generated by non-enzymatic glycation and oxidation of proteins and reducing sugars (Bierhaus et al. 1997). AGEs can induce oxidative stress in cells bearing AGE receptors (Lieuw-a-Fa et al. 2006). These observations have led to the hypothesis that glycation-induced microvascular complications result from a cycle of AGE-dependent oxidative stress (Fu et al. 1998). AGEs have been implicated as causal factors in endothelial dysfunction associated with vascular diseases. They accumulate during aging and at an accelerated rate in diabetes (Schleicher et al. 1997).

N^{ε}-carboxymethyllysine (CML) is a major AGE product formed by the combined reactions of non-enzymatic glycation and oxidation (glycoxidation) (Reddy et al. 1995; Fu et al. 1996). Subsequent studies indicate that CML

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plays an important role in the pathogenesis of heart disease (Nerlich and Schleicher 1999; Schalkwijk et al. 2004; Kralev et al. 2009). Intracellular concentrations of CML reflect endothelial dysfunction by integrated oxidative stress over long periods of time (Hammes et al. 1999). Although enhanced accumulation of CML in renal tissue, dermis and blood vessels in diabetic and aging individuals has been reported (Schleicher et al. 1997), the disease- and/or age-associated changes in CML levels in cardiac tissues remain to be elucidated. CML plays a role in the local lesions, and it is not enough to just rely on the circulating CML, but must rely on the local CML. The presence of CML in the local lesions may be derived from deposition of the circulating CML as well as local generation. Therefore, investigations of circulating CML levels in the plasma or serum alone are insufficient for accurate evaluation the role of CML in the pathogenesis of heart disease. Based on such considerations, in this study we first obtained cardiac tissues from patients undergoing cardiac surgery, and investigated changes in the CML levels in cardiac tissues to more fully elucidate the role of CML in the pathogenesis of heart disease.

Methods

Study group and clinical data

The study was performed on 105 consecutive patients undergoing cardiac surgery from November 2011 to November 2012 (55.6 ± 17.0 years old; age range, 1-78 years), and it is in accordance with the approval of the Southeast University Medical Ethics Committee (Approval No: 2012ZD11KY28.0). Patients' sex and age, smoking status, blood pressure (BP), disease type and medication use were recorded. Fasting total cholesterol (TDL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), super sensitivity C-reactive protein (sCRP) and renal function were measured in an accredited laboratory. The renal function was evaluated with endogenous creatinine clearance rate (Ccr): Ccr = [(140 − age) × weight (kg)]/[72 × Scr (mg/dl)] [×0.85 (if female)]. Scr stands for serum creatinine.

Quantitative measurements

A 5 ml blood sample was collected from each patient between 6:00 and 7:00 a.m. after an overnight fast (12-14 h) into EDTA (1 mg/ml). The sample was centrifuged at 3,000 r/min for 15 min at room temperature within 90 min of collection, and the supernatant was stored at −80°C for the CML assay.

Human cardiac tissue samples (0.02-0.5 g) were collected from tissues removed during cardiac surgery. In general, the surgical waste comprised heart auricles (appendix of the atrium), which were approximately 0.4-2.0 g. Immediately after surgical removal, cardiac tissue samples (auricles) were obtained and weighed, rinsed in ice cold saline to remove blood, wiped with filter paper, reweighed and minced. After adding cold 0.86% NaCl solution, tissues were ground into 10% homogenates with a tissue homogenizer (10,000-15,000 r/min for 10 s/time (total, 3-5 times) at 30 s intervals, operating on ice water). Prepared 10% homogenates were centrifuged for 10 to 15 min (3,000 r/min), and the supernatant were stored at −80°C for later CML assays.

CML concentrations were determined using a CML ELISA kit (Westang Bio-tech, Shanghai, China, Catalog No: F00438). A standard curve for the determination of CML concentrations in plasma samples was constructed using serial dilutions of a CML standard preparation (4,000, 2,000, 1,000, 500, 250, 125, 62.5 and 0 ng/mL). Microtitre plates were pre-coated with the CML monoclonal antibody. CML standard preparation or plasma samples (100 μL) were added to the microtitre plate in duplicate and incubated for 40 min at 37°C. Subsequently, the biotinylated anti-human CML antibody was added, followed by horseradish peroxidase-labelled streptavidin. Immunoreactivity was visualized using the substrate (TMB Solution). The reaction was stopped by the addition of stop solution (sulfuric acid). The OD value measured at 450 nm is proportional to the CML concentration. All steps were performed according to manufacturer's protocols and the CML concentration (ng/mL) in the sample was determined from the standard curve.

Statistical analysis

Data analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and CurveExpert 1.3 (Daniel G. Hyams, Crossgate Street Starkville, MS, USA). Data were presented as mean ± S.D. (standard deviation) or number and percentage for categorical variables unless otherwise stated. A p-value of < 0.05 (two-tailed) was considered statistically significant. A logistic model was used to calculate the CML concentration. Pearson correlation was used to test the association of two parameters. To describe the linear association of the concentration of CML in cardiac tissue and a set of exploratory variables, a multiple linear regression model was developed by the stepwise method.

Results

Clinical characteristics of study population

Of 105 consecutive patients, with a mean age of 55.6 ± 17.0 years (range, 1-78 years), the majority was male (59, 56.2%). The major diseases identified among these patients were CHD (31, 29.5%), CHD associated with DM (19, 18.1%), valvular heart disease (43, 41%) and congenital heart disease, such as atrial septal defect, ventricular septal defect, (12, 11.4%). Serum creatinine was 84.0 ± 32.8 (61-194) mg/dl, Ccr was 72.6 ± 22.8 (28.6-139.1) ml/min/1.73 m^2, and sCRP was 18.1 ± 31.3 (0.19-148) mg/L. New York Heart Association (NYHA) functional classification included class I to IV. The plasma CML concentration was 341.6 ± 135.8 (60.1-580.3) ng/ml. Detailed clinical information of the study subjects is shown in Table 1, and the data for each disease are shown in Table 2.

Cardiac tissue CML concentrations and correlation with clinical characteristics

The concentration of CML in cardiac tissues among all the patients was 3.55 ± 1.46 (0.36-6.03) μg/g, and cardiac CML levels with respect to disease were 4.31 ± 0.66, 5.29 ± 0.59, 2.74 ± 1.05 and 1.75 ± 1.16 μg/g, respectively (Table 2). Multiple linear regression analysis showed that age, DM, CHD were three mutually independent factors, and cardiac CML levels had a significant positive correlation with them (r values: 0.803 ($p < 0.001$), 0.567 ($p < 0.001$) and 0.523 ($p < 0.001$), respectively). R^2 was 0.872 ($p <$

Table 1. Baseline characteristics of the study population (n=105).

Age (years)	55.6 ± 17.0
Male	59 (56.2%)
Smoker	44 (41.9%)
NYHA: I or II	42 (40%)
NYHA: III or IV	63 (60%)
Systolic BP (mmHg)	131.3 ± 24.1
Diastolic BP (mmHg)	80.3 ± 14.9
TDL-cholesterol (mmol/L)	4.27 ± 1.27
HDL-cholesterol (mmol/L)	1.16 ± 0.37
LDL-cholesterol (mmol/L)	2.65 ± 1.00
sCRP (mg/L)	18.1 ± 31.3
Ccr (ml/min/1.73 m^2)	77.6 ± 22.8
Coronary heart disease	50 (47.6%)
Diabetes	19 (18.1%)
Hypertension	44 (41.9%)
Medications	
Beta-blocker	68 (64.8%)
ACE-i/ARB	72 (68.6%)
Spironolactone	60 (57.1%)
CCB	45 (42.9%)
Statins	50 (47.6%)
diuretic agents	63 (60%)
CML in Plasma (ng/ml)	341.6 ± 135.8
CML in heart (μg/g)	3.55 ± 1.46

Data are mean ± S.D., n (%).

ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; sCRP, super sensitivity C-reactive protein; Ccr, endogenous creatinine clearance rate; NYHA, New York Heart Association; CCB, calcium channel blocker; CML, N^{ε}-(Carboxymethyl)lysine.

0.001), indicating that the three independent variables could explain 87.2% variation of CML concentrations (Fig. 1).

Univariate linear analysis showed a significant correlation between Ccr and the concentration of CML (r = −0.451, p = 0.001), but this relationship was lost after adjustment for age (r = −0.075, p = 0.618). The CML levels of the non-smoking and smoking groups were 2.98 ± 1.50 μg/g and 4.35 ± 0.95 μg/g ($p < 0.01$), respectively. Detailed clinical data for smoking are shown in Table 3. After adjusting for age, the CML level still exhibited a significant correlation with smoking (r = 0.232, p = 0.018). The CML levels of the non-hypertension and hypertension groups were 2.95 ± 1.37 μg/g and 4.39 ± 1.14 μg/g ($p < 0.01$) respectively. Detailed clinical data for hypertension are shown in Table 4. After adjusting for age, the CML level still exhibited a significant correlation with hypertension (r = 0.340, $p < 0.01$). However, coupled with disease factors, multivariate regression analysis showed that the impact of smoking and hypertension on CML levels was lost (r = 0.076 (p = 0.454) and 0.120 (p = 0.234), respectively).

No correlations were found with other clinical characteristics, such as NYHA, BP, TDL, HDL, LDL and sCRP. Furthermore, it was identified that the use of agents such as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), beta-adrenergic antagonists, calcium-channel blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and diuretic agents had no effects on cardiac CML concentrations.

Plasma CML concentrations among all the patients were 341.6 ± 135.8 (60.1-580.3) ng/ml. The plasma CML concentrations with respect to disease (Table 2) exhibited a significant positive correlation with age, DM and CHD.

Table 2. Detailed clinical information for each disease of the study population (n = 105).

	CHD (n = 31)	CHD with DM (n = 19)	Valvular heart disease (n = 43)	Congenital heart disease (n = 12)
Age (years)	65.1 ± 9.3	62.9 ± 10.0	52.8 ± 14.4	29.9 ± 21.4
Male	23 (74.2%)	14 (73.7%)	16 (37.2%)	6 (50%)
Smoker	22 (71%)	11 (57.9%)	9 (20.9%)	2 (16.7%)
NYHA: I or II	12 (38.8%)	12 (63.2%)	12 (27.9%)	6 (50%)
NYHA: III or IV	19 (61.1%)	7 (36.9%)	31 (72.1%)	6 (50%)
Systolic BP (mmHg)	135.5 ± 23.4	137.2 ± 27.1	128.7 ± 25.4	118.2 ± 18.9
Diastolic BP (mmHg)	83.4 ± 15.0	82.8 ± 13.5	77.7 ± 14.9	76.9 ± 16.0
TDL-cholesterol (mmol/L)	4.24 ± 1.12	4.32 ± 1.17	4.40 ± 1.50	3.74 ± 0.87
HDL-cholesterol (mmol/L)	1.07 ± 0.24	1.06 ± 0.24	1.25 ± 0.48	1.28 ± 0.39
LDL-cholesterol (mmol/L)	2.66 ± 0.98	2.69 ± 0.82	2.78 ± 1.10	2.10 ± 0.86
sCRP (mg/L)	16.9 ± 30.3	25.0 ± 36.0	18.9 ± 32.7	6.5 ± 15.1
Ccr (ml/min/1.73 m^2)	78.4 ± 25.0	65.1 ± 18.3	79.6 ± 23.8	87.0 ± 14.4
CML in Plasma (ng/ml)	413.3 ± 64.9	505.9 ± 54.5	263.9 ± 91.9	174.9 ± 103.4
CML in heart (μg/g)	4.31 ± 0.66	5.29 ± 0.59	2.74 ± 1.05	1.75 ± 1.16

Data are mean ± S.D., n (%).

NYHA, New York Heart Association; sCRP, super sensitivity C-reactive protein; Ccr, endogenous creatinine clearance rate; CML, N^{ε}-(Carboxymethyl)lysine; DM, diabetes mellitus; CHD, coronary heart disease.

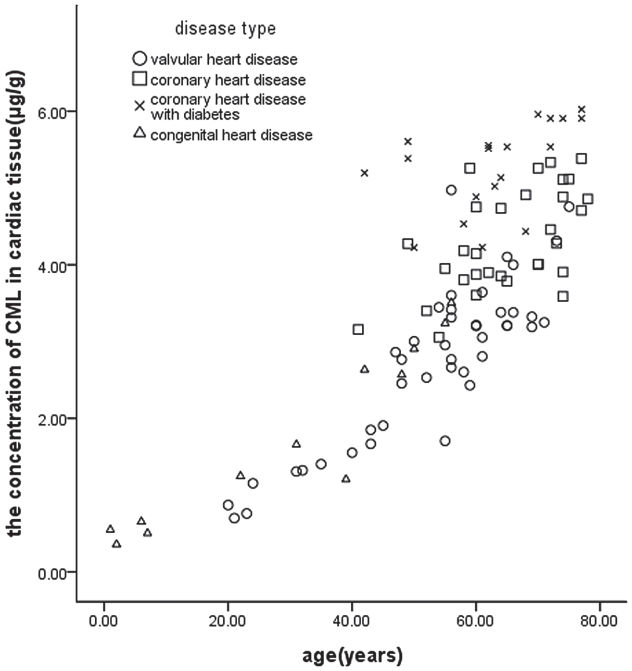

Fig. 1. Association between age, disease type and cardiac CML concentrations (n = 105).
Multiple linear regression analysis showed that all four diseases (coronary heart disease (CHD), CHD associated with diabetes mellitus (DM), valvular heart disease and congenital heart disease) had significant impact on cardiac CML concentrations, but after adjustment for age the concentration of CML in cardiac tissues exhibited a significant positive correlation only with age (r = 0.803, p < 0.001), DM (r = 0.567, p < 0.001) and CHD (r = 0.523, p < 0.001), which together explain 87.2% variation in cardiac CML concentrations (p < 0.001).

The corresponding r values were 0.769 (p < 0.001), 0.553 (p < 0.001) and 0.544 (p < 0.001), respectively, while R^2 of 0.861 (p < 0.001) suggested that the three independent variables could explain 86.1% variation of CML concentrations (Fig. 2). Similarly, univariate linear analysis showed a significant correlation between Ccr and CML concentrations (r = −0.458, p = 0.001), but after adjusting for age, the relationship was also lost (r = 0.099, p = 0.507). Linear regression analysis indicated that cardiac CML concentrations exhibited a highly significant positive correlation with plasma CML concentrations (r = 0.983, p < 0.001) (Fig. 3).

Discussion

This is the first study investigating the concentration of CML in human cardiac tissues. Our results demonstrated that cardiac CML concentrations increase with age, and further increase in patients with DM and/or CHD. Furthermore, we demonstrated that the changes in cardiac CML concentrations are consistent with those in plasma.

CML is a major AGE product (Reddy et al. 1995). AGEs are represented by a heterogeneous group of molecules formed by the non-enzymatic reaction of reducing sugars, ascorbate, and other carbohydrates with amino acids, nucleic acids, and lipids (Fu et al. 1996). AGEs can lead to vascular damage by forming intra- and inter-molecular cross-links with matrix proteins in the vascular wall, which increase vessel rigidity, trap lipoproteins within the arterial wall and disrupt the clearance of the vessel (Eble et al. 1983). Furthermore, AGEs interact with the endothelial receptor of AGE (RAGE), resulting in the induction of procoagulant activity, impairment of endothelium-dependent relaxation, increased vascular permeability, migration of

Table 3. Detailed clinical information for smoking of the study population (n=105).

	Smoking (n = 44)	Non-smoking (n = 61)	p value
Age (years)	64.2 ± 9.4	49.5 ± 18.6	< 0.01
Systolic BP (mmHg)	134.8 ± 24.7	128.7 ± 25.0	0.22
Diastolic BP (mmHg)	80.0 ± 15.5	80.4 ± 14.5	0.89
TDL-cholesterol (mmol/L)	4.42 ± 1.28	4.2 ± 1.27	0.31
HDL-cholesterol (mmol/L)	1.15 ± 0.34	1.17 ± 0.41	0.79
LDL-cholesterol (mmol/L)	2.74 ± 1.05	2.57 ± 0.95	0.41
sCRP (mg/L)	19.3 ± 34.3	17.3 ± 29.0	0.76
Ccr (ml/min/1.73 m^2)	69.5 ± 22.0	82.8 ± 22.1	< 0.05
Disease type			
CHD	22 (50%)	9 (14.8%)	—
CHD with DM	11 (25%)	8 (13.1%)	—
Valvular heart disease	9 (20.5%)	34 (55.7%)	—
Congenital heart disease	2 (4.5%)	10 (16.4%)	—
Hypertension	25 (56.8%)	19 (31.1%)	—
CML in Plasma (ng/ml)	414.5 ± 93.0	289.1 ± 138.1	< 0.01
CML in heart (μg/g)	4.35 ± 1.46	2.97 ± 1.50	< 0.01

Data are mean ± S.D., n (%).

sCRP, super sensitivity C-reactive protein; Ccr, endogenous creatinine clearance rate; CHD, coronary heart disease DM, diabetes mellitus; CML, N^{ε}-(Carboxymethyl)lysine.

Table 4 Detailed clinical information for hypertension of the study population. (n=105).

	Hypertension (n=44)	Non- hypertension (n=61)	p value
Age (years)	63.2 ± 10.1	50.2 ± 18.9	< 0.01
Systolic BP (mmHg)	145.4 ± 25.3	120.8 ± 18.8	< 0.01
Diastolic BP (mmHg)	86.9 ± 16.3	75.3 ± 11.5	< 0.01
TDL-cholesterol (mmol/L)	4.04 ± 1.13	4.44 ± 1.36	0.12
HDL-cholesterol (mmol/L)	1.06 ± 0.32	1.23 ± 0.39	<0.05
LDL-cholesterol (mmol/L)	2.46 ± 0.82	2.79 ± 1.10	0.13
sCRP (mg/L)	20.9 ± 35.5	16.1 ± 27.7	0.46
Ccr (ml/min/1.73 m^2)	75.6 ± 25.5	79.4 ± 20.4	0.57
Disease type			
CHD	19 (43.2%)	12 (19.7%)	—
CHD with DM	14 (31.8%)	5 (8.2%)	—
Valvular heart disease	11 (25%)	32 (52.5%)	—
Congenital heart disease	0	12 (19.7%)	—
CML in Plasma (ng/ml)	420.9 ± 110.6	284.5 ± 123.5	< 0.01
CML in heart (μg/g)	4.39 ± 1.14	2.95 ± 1.37	< 0.01

Data are mean ± S.D., n (%).

sCRP, super sensitivity C-reactive protein; Ccr, endogenous creatinine clearance rate; CHD, coronary heart disease; DM, diabetes mellitus; CML, N^{ε}-(Carboxymethyl)lysine.

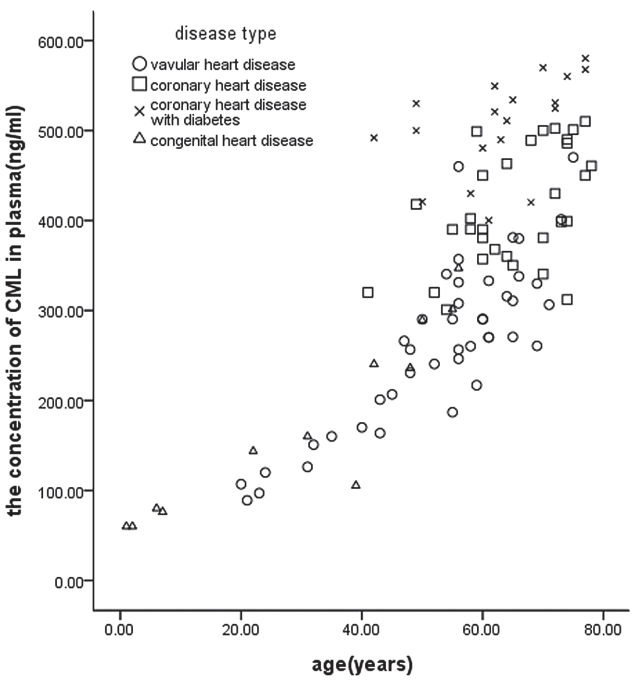

Fig. 2. Association between age, disease type and plasma CML concentrations (n = 105).
Multiple linear regression analysis showed that all four diseases (coronary heart disease (CHD), CHD associated with diabetes mellitus (DM), valvular heart disease and congenital heart disease) had significant impact on plasma CML concentrations, but after adjustment for age the CML concentration exhibited a significant positive correlation only with age (r = 0.769, p < 0.001), DM (r = 0.553, p < 0.001) and CHD (r = 0.544, p < 0.001), which together explain 86.1% variation in plasma concentrations (p < 0.001).

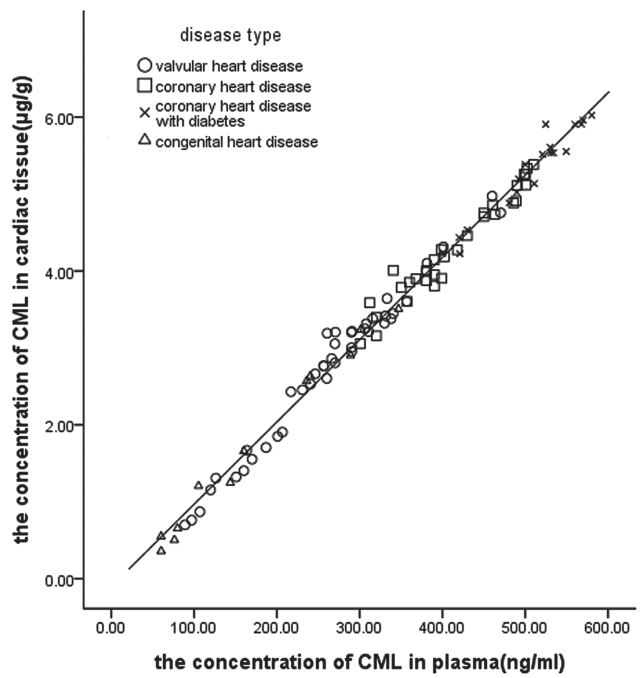

Fig. 3. Correlation between cardiac and plasma CML concentrations (n = 105).
Linear regression analysis indicated that cardiac CML concentrations exhibited a highly significant positive correlation with plasma CML concentrations (r = 0.983, p < 0.001).

macrophages, and T-lymphocytes into the intima and oxidative stress (Peppa et al. 2002; Peppa et al. 2004). One of the best characterized AGEs is CML (Reddy et al. 1995). CML adducts are ligands for RAGE, which activate cell signaling pathways, such as extracellular signal-regulated

kinases (ERK1/ERK2) and nuclear factor-κB (NF-κB) that modulate expression of genes including vascular cell adhesion molecule-1 on endothelial cells, mononuclear phagocytes, and vascular smooth muscle cells (Stitt et al. 1997; Lander et al. 1997; Kislinger et al. 1999; Hammes et al. 1999; Kislinger et al. 2001; Yeh et al. 2001; Ballinger et al. 2005).

It has been proposed that the principal mechanism underlying the degradation of AGEs modified tissues and cells involves specific AGE receptors expressed on tissue macrophages (Makita et al. 1991). After degradation small soluble AGE peptides are released and subsequently cleared by the kidney. Therefore, effective elimination is dependent on normal creatinine clearance, and any deterioration in renal function can result in AGEs accumulation leading to endothelial perturbation and hence, vascular disease (Bierhaus et al. 1998). Chronic kidney disease (CKD) is an independent risk factor for cardiovascular disease outcomes, particularly in higher-risk populations (Sarnak et al. 2003; McCullough et al. 2007). However, in the present study patients with renal insufficiency were few, and therefore, pairing by age was impossible. Furthermore, the small number of patients with renal insufficiency appeared with increasing age. Therefore, further evaluation of Ccr was not conducted.

The predominant forms of heart disease in the elderly are CHD and HF. Their common pathological changes consist of vascular lesions at all levels resulting in the decline of oxygen and other nutrients required by myocardial cells in the corresponding area. This leads to a decline in the functional capacity and even death of myocardial cells, which are replaced by myocardial fibrosis, causing cardiac function to decline gradually. It has been reported that CML deposition is localized in the small intramyocardial arteries in endothelial cells and smooth muscle cells, but not in myocardial cells (Schalkwijk et al. 2004). CML has also been detected in atherosclerotic lesions, and is thought to be an endogenous biomarker of local oxidative stress (Nerlich and Schleicher 1999). Plasma AGEs, in particular CML levels, are related to the severity and prognosis of HF (Hartog et al. 2007).

AGEs can covalently crosslink and biochemically modify protein structure, and affect protein functions, particularly collagen (Brownlee et al. 1988), as well as the structural components of the extracellular matrix (Sell et al. 1992; Beisswenger et al. 1995). Cardiac AGEs accumulation is associated with a significant decrease in myocardial collagen solubility, which is an index of increased collagen crosslinking (Candido et al. 2003). Evidence has been presented that AGEs formation associated with DM reduces extracellular matrix degradation and angiogenesis (Tamarat et al. 2003). Stimulation of AGEs receptors by AGEs leads to (prolongs) cellular activation and release of inflammatory cytokines. These changes may result in the development and progression of diastolic and systolic dysfunction, and subsequent HF (Aronson 2003; Zieman and Kass 2004). It has been reported that aging, CHD and DM all affect the structure of the heart, for example, the loss of cardiac myocytes, cardiac myocyte hypertrophy, and collagen deposition in the extracellular matrix, resulting in myocardial interstitial fibrosis and reduced cardiac function (Olivetti et al. 1991; Thomas et al. 1992; Weber et al. 1993; Avendano et al. 1999; Burgess et al. 2001; Liu et al. 2003). Aminoguanidine specifically prevents the formation of AGEs, and ALT-711 is an AGE crosslink breaker. Both of these agents have been reported to reverse the cardiovascular rigidity associated with aging and diabetes (Huijberts et al. 1993; Norton et al. 1996; Wolffenbuttel et al. 1998; Asif et al. 2000; Kass et al. 2001; Candido et al. 2003). Therefore, it can be concluded that CML plays an important role in the pathogenesis of heart disease.

This study found that the cardiac tissue CML concentrations increased with age, and were still significantly higher in patients with CHD after adjusting for age. All patients with DM in the study group were complicated with CHD. After adjusting for age and CHD, the cardiac CML concentrations in diabetic patients were still significantly higher. CML generation and deposition gradually increase with age, leading to gradual enhancement of its harmful effects. Therefore, the incidence of heart disease increases with age. In diabetic patients, the local CML concentrations in cardiac tissues are much greater than those detected in non-diabetic patients; therefore, in diabetic patients, the age of onset of heart disease is younger and the incidence is higher. Although the local CML deposition mechanism is complex and not yet fully elucidated, in general, it can be classified into three types: 1) Increased local CML generation; 2) Increased levels of circulating CML and deposition; 3) Decline in metabolic clearance of CML. In diabetic patients, the predominant mechanism may be increased local deposition caused by increases in both circulating CML levels and local generation. In older patients, the main mechanism may be the long-term deposition of small amounts of CML and the decline in metabolic clearance. Compared with the elderly unaffected by heart disease and DM, the cardiac CML concentrations in the elderly suffering from heart disease alone further increase, and it is likely to be accompanied by an increase in local CML generation due to mechanisms such as local oxidative stress (Fu et al. 1998). Therefore, the concentration of CML in cardiac tissues exhibits a close correlation with heart disease.

In conclusion, the concentration of CML in cardiac tissues increases with age, especially in patients with DM and/or CHD. Furthermore, the plasma CML concentration may be a promising tool for assessment of the concentration of CML in cardiac tissues. We speculate that CML is linked to the pathogenesis of CHD and HF, especially various heart diseases associated with DM. This study provides the basis for further investigations to elucidate the exact role of CML in the pathology of heart disease.

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Conflict of Interest

The authors declare no conflict of interest.

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