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Original article

Relationship between cardiac tissue glycation and skin autofluorescence in patients with coronary artery disease

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Abstract

Aim. – During ageing, advanced glycation end-products (AGEs) accumulate in extracellular matrix proteins like collagen and contribute to a decline in organ function. As skin autofluorescence (sAF) can assess subcutaneous accumulation of fluorescent AGEs, this study aimed to investigate the relationship between AGE-modified cardiac tissue collagen and AGE-related sAF in coronary artery bypass graft (CABG) surgery patients.

Methods. – Between January 2011 and January 2012, data from 72 consecutive male patients undergoing isolated CABG were prospectively recorded. Collagen fractions were isolated from the right atrial appendages of these patients by proteolysis and collagenase digestion. Collagen was quantified by hydroxyproline assay, and AGEs by AGE-related intrinsic fluorescence; sAF was measured using an autofluorescence reader.

Results. – Biochemical analysis showed that the insoluble cardiac collagen fraction contained the highest amounts of accumulated AGEs; the AGE-related intrinsic fluorescence of this fraction increased with age ($P = 0.0001$), blood glucose ($P = 0.002$), HbA_{1c} ($P = 0.01$) and sAF ($P = 0.008$).

Conclusion. – This study demonstrated for the first time a relationship between cardiac tissue glycation and AGE-related sAF. In addition, cardiac tissue glycation was associated with age, blood glucose and long-term glucose values in patients with coronary artery disease.

Keywords: AGEs; Coronary artery disease; Diabetes mellitus; Skin autofluorescence

1. Introduction

Ageing is defined by a progressive decline in the physiological and biochemical function of tissues and organs resulting in degenerative diseases [1]. Advanced glycation end-products (AGEs) appear to be involved as a basic mechanism of ageing as well as in the development of age- and/or diabetes-related diseases, such as cardiovascular disease. AGEs are the irreversible result of the Maillard reaction, which starts by non-enzymatic modification of protein by sugars. Initially, this is a reversible reaction that leads to the formation of unstable compounds, the so-called Schiff base. The latter undergoes molecular rearrangements over a period of days, yielding more stable Amadori products. Over weeks/months, the Amadori products turn, through a series of reactions such as oxidation, into very stable compounds known as AGEs. These are formed in extracellular matrix proteins like collagen, laminin and elastin, which alter the physiological properties of the matrix and increase its stiffness [2]. Ageing of the heart is characterized by loss of myocytes and proliferation of fibroblasts, which produce collagen. The accumulation of interstitial collagen alters the physiological properties of the ageing heart, leading to fibrosis, left ventricular hypertrophy and diastolic dysfunction [3,4].

Abbreviations: AGEs, advanced glycation end-products; AIF, AGE-related intrinsic fluorescence; a.u., arbitrary units; CAD, coronary artery disease; CABG, coronary artery bypass grafting; CDCF, collagenase digestible collagen fraction; HbA_{1c}, glycated haemoglobin; ICF, insoluble collagen fraction; PDCF, pepsin digestible collagen fraction; sAF, skin autofluorescence.

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Tissues rich in extracellular matrix and long-lived proteins such as the vessels and the heart are affected by AGE modifications, including glycation, glycoxidation and cross-linking [5,6]. Indeed, the cross-linking due to AGEs is believed to further increase the loss of tissue elasticity, resulting in rigidity and deterioration of heart function with age [7,8]. This process modulates collagen quality [9]. AGEs also contribute to endothelial dysfunction by enhancing oxidative stress and, due to activation of their receptors (RAGEs), they also accelerate proinflammatory atherosclerotic processes [10,11].

It is known that plasma levels of AGEs are influenced by nutrition and may therefore not truly reflect tissue AGE accumulation [6,12], so the measurement of skin autofluorescence (sAF) to estimate tissue AGE burden is widely established [13,14]. It has been shown that non-invasive sAF correlates well with cardiac mortality in diabetic and haemodialysis patients [14–19]. Recently, sAF was reported to be a strong predictor of vascular AGE modifications and to alter vascular function [20]. However, to date, the determination of tissue AGEs has mainly been based on easily accessible skin biopsies, and it is not known whether AGE-modified collagen fractions of the heart correlate with sAF measurements. Also of interest is to determine which cardiac tissue collagen fraction correlates with age and diabetes. The present prospective study therefore tested whether cardiac tissue AGE accumulation is related to age, diabetes and sAF in patients scheduled for elective coronary artery bypass graft (CABG) surgery.

2. Patients and methods

2.1. Preoperative patient characteristics

Between January 2011 and January 2012, data from 72 consecutive male Caucasian patients undergoing elective first-time isolated CABG surgery were prospectively recorded. The focus was on males, as the number of females with isolated CABG surgery in our unit was too small. The right atrial appendage was obtained from these patients. At the preoperative visit, all study parameters including sAF were determined. Fasting blood samples were collected from the cubital vein between 7 am and 8 am on the day of the operation.

The study was approved by the local medical ethics committee and was carried out in accordance with the Declaration of Helsinki guidelines. Written informed consent was obtained from all study participants.

2.2. Tissue preparation and collagen extraction

The method used has been described elsewhere [20,21]. From the right atrial appendage, collagen types I and III were isolated by pepsin digestion and collected as the pepsin digestible collagen fraction (PDCF). The residual pellet was rinsed and digested with collagenase type I and proteinase K and, after centrifugation, the supernatant was considered the collagenase digestible collagen fraction (CDCF). The final pellet was acid hydrolysed. After evaporation, the dried sample was considered the insoluble collagen fraction (ICF).

2.3. Quantification of collagen

Collagen was estimated by measuring the amount of hydroxyproline. According to the literature, it may be assumed that hydroxyproline makes up 14% of collagen by weight. Collagen in each fraction was quantified by 4-hydroxyproline assay according to the method of Lin and Kuan [22].

2.4. AGE quantification in collagen fractions

AGE intrinsic fluorescence was measured in duplicate in all collagen fractions at 360 nm excitation and 440 nm emission with a plate reader (FLUOstar OPTIMA, BMG LABTECH, Offenburg, Germany), as described previously [20]. The measured fluorescence was normalized to the collagen concentration of the isolated collagen fractions.

2.5. Skin autofluorescence

The sAF was assessed using a validated sAF reader (AGE Reader, DiagnOptics, Groningen, Netherlands) as previously described [13,20]. Three repeated measurements were taken at a healthy pale skin site (no scars, hyperpigmentation or other skin abnormalities) on the volar aspect of the forearm, and an average was calculated. sAF was measured in arbitrary units (a.u.), and automatically calculated by dividing the mean value of the emitted light intensity (per nm) between 420 and 600 nm by the mean value of the excitation light intensity (per nm) between 300 and 420 nm. Reproducibility was indicated by an intra-individual Altman error of 5.0% on a single day and 5.9% for seasonal changes [13].

2.6. Statistical analysis

Categorical variables were expressed as frequencies and percentages. Metric variables were expressed as means ± standard deviation (SD) or means ± standard error of the mean (SEM). Differences between groups were analysed using a *t* test and, when normality was not met, with the Mann–Whitney *U* test. Skewed variables were log-transformed prior to the analyses. P values < 0.05 were considered significant.

3. Results

3.1. Baseline characteristics

In the present study, 72 male patients diagnosed with coronary artery disease (CAD) and scheduled for CABG surgery were assessed. All patients were taking aspirin and beta-blockers; 93% were taking angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (AT1). Fourteen patients with type 2 diabetes (T2D) were taking oral agents alone and eight were being treated with oral agents plus insulin.