Atherosclerosis 252 (2016) 88–96

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Altered relation of the renin-aldosterone system and vasoactive peptides in type 2 diabetes: The KORA F4 study

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ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form
14 July 2016
Accepted 15 July 2016
Available online 17 July 2016

Keywords:
Diabetes
Renin
Aldosterone
Adrenomedullin
ANP
MR-proANP
MR-proADM

ABSTRACT

Background and aims: The exact mechanism of premature atherosclerosis in diabetes is still unclear. Inappropriate activation of the renin-aldosterone-angiotensin system may be an important risk factor for cardiovascular disease. We investigated whether renin and aldosterone are associated with vasoactive peptides midregional-pro atrial natriuretic peptide (MR-proANP) and midregional-pro adrenomedullin (MR-proADM), or with intima media thickness (IMT) as a marker for early atherosclerotic alterations in the general community and in subjects with type 2 diabetes.

Methods: In 1261 participants in the KORA F4 study, the associations of renin, aldosterone and aldosterone to renin ratio with MR-proANP, MR-proADM and IMT were assessed using linear regression models stratified for the presence of prediabetes and type 2 diabetes.

Results: After adjustment for confounding factors, an inverse association of MR-proANP with renin ($p = 0.002$) and aldosterone ($p = 0.021$) and a direct association of MR-proADM with renin ($p < 0.001$) and aldosterone ($p = 0.019$) were seen in nondiabetic individuals. In diabetic subjects, there was no significant correlation of MR-proANP or MR-proADM with renin or aldosterone. Renin and aldosterone were not directly associated with IMT in non-diabetic subjects and the total cohort, whereas aldosterone was associated with IMT in diabetic participants ($p = 0.005$).

Conclusions: This study shows associations between renin, aldosterone and MR-proANP/MR-proADM plasma levels that are altered in type 2 diabetes. Plasma renin and aldosterone are not independent biomarkers for early atherosclerotic damages of the carotid arteries in the general community.

The renin-angiotensin-aldosterone system (RAAS) plays a key role in body fluid and blood pressure homeostasis [1]. Inappropriate RAAS activation is a well-known cardiovascular risk factor [2,3]. In addition to causing hypertension by volume overload and vasoconstriction, angiotensin II, the principal RAAS effector peptide, and aldosterone are directly involved in vascular inflammation, remodeling and fibrosis [4]. The direct involvement of renin in cardiovascular disease is less clear. However, renin has also been associated with cardiovascular events independently of its downstream effectors [5,6].

Potential opponents of the RAAS are peptides with vasodilatative properties, such as natriuretic peptides and adrenomedullin. Natriuretic peptides exert relaxant effects on vascular smooth muscle cells [7], reduce the sympathetic tone, inhibit the secretion of the vasoconstrictor endothelin-1 [8] and may also suppress the RAAS [9]. In addition, natriuretic peptides have direct

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http://dx.doi.org/10.1016/j.atherosclerosis.2016.07.905
0021-9150/

protective anti-hypertophic and anti-fibrotic cardiovascular effects [10]. Release of atrial natriuretic peptide (ANP) from the atria is mediated in response to atrial distension by volume overload [11], whereas brain natriuretic peptide (BNP) is mainly secreted by ventricular myocytes following ventricular wall stress. ANP is the predominant natriuretic peptide in physiological conditions. BNP levels are normally low and only become notable in pathological conditions [3,12]. Adrenomedullin (AMD) is a 52 amino acid peptide with strong vasodilatative properties, which is synthesized by a variety of tissues, including adrenal medulla, vascular endothelial cells and vascular smooth muscle cells [13]. Both natriuretic peptides and adrenomedullin have a very short plasma half-time. Thus, reliable assays targeting the stable midregional-pro atrial natriuretic peptide (MR-proANP) and mid-regional proadrenomedullin (MR-proADM) have been developed. These stable prohormones are released in equimolar concentrations to the mature peptides. Circulating MR-proANP [14,15] and MR-proADM [13,15,16] have been shown to correlate with chronic heart failure, cardiovascular events [16–18] and components of the metabolic syndrome [19,20].

Assuming an interplay between vasoregulatory peptides and the RAAS, we sought to determine the association of renin, aldosterone and aldosterone to renin ratio (ARR) with the vasodilatory hormones MR-proANP and MR-proADM and with common carotid intima-media thickness (IMT) representing an established marker of early vascular damage that predicts future cardiovascular complications independently of traditional risk factors [21,22]. We were particularly interested in the possible interplay of vasoregulatory peptides and RAAS in diabetes and prediabetes. Disturbed glucose tolerance is a major cardiovascular risk factor. However, the exact mechanisms of premature atherosclerosis in diabetes still remain unclear. We hypothesized that dysregulation of vasoactive hormones may contribute to endothelial dysfunction in subjects with disturbed glucose tolerance. Therefore, we stratified the study cohort for the presence of diabetes and prediabetes.

1. Materials and methods

1.1. Study participants

The KORA (Cooperative Health Research in the Region of Augsburg, southern Germany) F4 study is a population-based cohort of 3080 subjects recruited between 2006 and 2008 (follow-up study of the KORA S4 survey conducted in 1999–2001). The study design, standardized sampling methods and data collection (medical history, medication, anthropometric measurements, blood pressure) have been described in detail elsewhere [23,24]. All study participants gave written informed consent and the study was approved by the Ethics Committee of the Bavarian Medical Association. From this cohort, a sample of 1596 subjects was randomly selected for plasma MR-proANP and MR-proADM measurements. Among those, IMT measurement was performed in 1298 subjects. All variables required for the current analyses were available in 1261 study participants. Criteria for a diagnosis of diabetes were a validated physician's diagnosis or current use of glucose-lowering agents. After an overnight fasting period, all non-diabetic participants underwent a standard 75 g oral glucose tolerance test. Newly diagnosed diabetes, impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and normal glucose tolerance (NGT) were defined according to the 1999 World Health Organization diagnostic criteria [25] based on both fasting and post-challenge glucose values (type 2 diabetes: $\geq$ 7.0 mmol/l fasting and/or $\geq$ 11.1 mmol/l 2-h glucose; IFG: $\geq$ 6,1 mmol/l and <7,0 mmol/l; IGT: $\geq$ 7.8 - < 11.1 mmol/l 2-h glucose). Prediabetes was defined as IFG and/or IGT (2-h glucose $\geq$ 7.8 - < 11.1 mmol/l). Hypertension was defined as systolic blood pressure $\geq$140 mmHg, diastolic blood pressure $\geq$90 mmHg or known hypertension with use of antihypertensive drugs.

1.2. Ultrasound and measurement of IMT

Ultrasound measurement (Sonoline G, 10-MHz transducer; Siemens Medical Solutions, Munich, Germany) of both common carotid arteries (CCA) was performed using a validated protocol [26] as previously described [27]. Optimal images of the right and left CCA far wall were recorded on DVD videotapes. IMT measurements were performed off-line over a length of 10 mm beginning at 0–5 mm of the dilatation of the distal CCA using an automated edge detection reading system (Prowin software, Medical Technologies International, USA). We used the average of the measurements of 3 frozen images from both the left and right CCA to calculate artery thickness of the distal CCA ((mean left + mean right)/2). One certified reader measured all IMT scans. Reproducibility studies for intersonographer (n = 30 IMT measurements) and inter-reader variability (n = 50 IMT measurements) revealed coefficients of variations of 1.9% and 3.0% with Spearman correlation coefficients of $\geq$0.89.

1.3. Laboratory measurements

Blood was collected after an overnight fast of at least 8 h without stasis, and the samples were kept at 4 °C until centrifugation. Except for 2-h glucose, all blood parameters were based on fasting blood samples. Plasma samples were stored at −80 °C until assayed. Measurements of blood glucose, HbA1c, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, serum creatinine and high sensitive C-reactive protein (hsCRP) were performed as described elsewhere [28]. Plasma aldosterone concentrations were measured with an in-house immunofluorescence assay and plasma renin levels were determined using an automated chemiluminescence immunoassay (LIASON Direct Renin, DiaSorin, Dietzenbach, Germany) as reported elsewhere [29]. ARR was calculated by dividing plasma aldosterone levels (ng/l) by plasma renin levels (ng/l). Glomerular filtration rate (eGFR) was calculated using the MDRD equation. Fasting insulin was determined by ELISA (Invitrogen, Karlsruhe, Germany). Plasma concentrations of MR-proANP and MR-proADM were measured after only one freeze-thaw cycle with an assay detecting the respective stable mid-regional precursor prohormone by sandwich fluoroimmunoassay (BRAHMS, Hennigsdorf/Berlin, Germany) using the automated system B.R.A.H.M.S KRYPTOR as described before [20,30]. The lower detection limit of this assay was 2.94 pmol/l for MR-proANP and 0.05 nmol/l for MR-proADM.

1.4. Statistical analyses

Clinical characteristics between subjects with and without type 2 diabetes/prediabetes were compared using F-tests in case of normally distributed variables. For log-normal variables, F-tests were performed on a log-scale. Logistic regression models were used to compare binomial proportions. Spearman correlation coefficients were calculated between MR-proANP/MR-proADM and renin, aldosterone and ARR, respectively.

Linear regression models were used to assess the association between quartiles of MR-proANP or MR-proADM as the independent variable and renin, aldosterone and ARR, respectively, as the dependent variables as well as the association of renin, aldosterone and ARR with IMT as dependent variable.

In linear regression analyses, the associations were assessed in four different models: (1) without adjustment, (2) with adjustment