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CD40–CD154 expression in calcified and non-calcified coronary lesions of patients with chronic renal failure

Valentina Campean[a,1], Daniel Neureiter[a,1], Barbara Nonnast-Daniel[b], Christoph Garlichs[c], Marie-Luise Gross[d], Kerstin Amann[a,*]

[a] *Department of Pathology, University of Erlangen-Nürnberg, Germany*
[b] *Internal Medicine IV, University of Erlangen-Nürnberg, Germany*
[c] *Internal Medicine II, University of Erlangen-Nürnberg, Germany*
[d] *Department of Pathology, Heidelberg, Germany*

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Abstract

The high incidence of cardiovascular complications in patients with chronic renal failure (CRF) is partly explained by more aggressive atherosclerosis, i.e. increased incidence and severity of lesions with higher tendency to calcification. The pathogenesis of this accelerated atherosclerosis, however, is not completely understood. Among other risk factors, chronic micro-inflammation may be involved. Activation of cells and adhesion molecules in atherosclerosis is governed by CD40–CD154 (CD40 ligand) interaction. Therefore, we investigated the expression and distribution of CD40–CD154 in different coronary atherosclerotic lesions of CRF patients and non-renal control patients.

Coronary plaques of 57 patients with and without CRF were categorized according to the Stary classification and analysed for in situ protein expression of CD40, CD154 and CRP using immunohistochemistry and a semiquantitative scoring system. The nature, number and distribution of infiltrating cells was analysed and correlated to the types of coronary lesions and in particular to the presence of calcification.

CD40 was over expressed in media myocytes of coronary plaques of both uremic and control patients. Inside the plaques, CD40 was expressed on endothelial cells, T lymphocytes, macrophages, fibroblasts, and smooth muscle cells. CD154 expression was seen on T cells in areas densely infiltrated by CD40 positive macrophages. In uremic and control patients higher in situ expression of CD40, CD154 and CRP was seen in calcified compared to non-calcified lesions. Inside the plaques, there were significant differences in the expression pattern of CD40 and CD154 between uremic and control patients. In addition, in uremic patients coronary plaques showed higher CRP protein expression compared to control patients.

The data indicate a higher inflammatory status of coronary lesions as well as involvement of the CD40–CD154 signaling cascade in CRF patients, especially in cases of calcified atherosclerotic lesions.

Keywords: Atherosclerosis; Chronic kidney disease; CD40; CD154; Inflammation

1. Introduction

Patients with chronic kidney disease (CKD) very early on develop severe atherosclerosis of coronary and peripheral arteries. As a consequences, cardiovascular complications are frequent and have a major impact on patients survival [1–4]. Clinical studies using X-ray and electron beam computer tomography (EBCT) [5,6] as well as histological studies

* Corresponding author at: Department of Pathology, University of Erlangen-Nürnberg, Krankenhausstr. 8–10, 91054 Erlangen, Germany. Tel.: +49 9131 8522291; fax: +49 9131 8522601.
E-mail address: kerstin.amann@patho.imed.uni-erlangen.de (K. Amann).
[1] Contributed equally to the work.

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[7,8] document an excessive high rate of vascular calcification in CKD patients. So far, only very limited information is available concerning the pathomechanisms of accelerated atherosclerosis, and in particular calcification.

In CKD patients the development and acceleration of atherosclerosis is not only linked to classical cardiovascular risk factors [9], but also associated with non-classical factors that may be a consequence of renal dysfunction [10,11]. Recently, micro-inflammation presently best reflected by C-reactive protein (CRP) has been identified as a potent predictor of cardiovascular death in renal patients with coronary heart disease [12]. Data from apolipoprotein E knockout-mice ($ApoE^{-/-}$) with renal failure as well as from vascular specimen of uremic patients provided evidences that oxidative stress may play a key role even in cases with only minor renal dysfunction [13,14]. In the serum of CKD patients biomarkers of inflammation like CRP and IL-6 are systemically elevated and this may cause endothelial cell dysfunction [15,16] and trigger an inflammatory response inside vascular structures through NF-kappaB [17]. Activation of NF-kappaB, however, is linked to the inflammation cascade system of CD40–CD154 (CD40-ligand, CD40L) [18,19]. Atherosclerosis is considered a localized inflammatory disease [20–22]. Recently, a direct atherogenic role of CRP was confirmed by Schwedler et al. who could show that injection of human CRP into $ApoE^{-/-}$ markedly aggravated atherosclerosis, local inflammation as well as local CD154 expression [23].

In non-renal patients activation of CD3-positive T lymphocytes, CD68-positive macrophages as well as CD40–CD154 interaction were involved in atherogenesis [24–27]. Detailed histological studies showed that expression of CD40–CD154 is associated with initiation and progression of atherosclerotic plaques [28,29]. These findings were confirmed by experimental data showing that CD154/ApoE double knock-out mice developed smaller atherosclerotic plaques than control mice [30]. Furthermore, specific antibody blockade of CD40–CD154 interaction was associated with a more stable plaque phenotype [31]. Additionally, in a recent study soluble CD40L was found to be a useful serological marker in patients at increased cardiovascular risk [32].

Overall, the role of the CD40–CD154 system and the inflammation inside atherosclerotic lesions of CKD patients are still under debate. Therefore, in the present study we investigated (i) the expression and distribution patterns of CD40–CD154 as well as (ii) the degree and the composition of the inflammatory cell infiltrate (by consecutive staining with CD3, CD20, CD31, CD68) in calcified and non-calcified coronary lesions of uremic and control patients.

It was the aim of the present study to assess and compare the expression and localization of the co-stimulatory CD40–CD154 ligand system in coronary plaques of CKD patients and non-renal controls. In addition, we aimed to investigate whether these expression and distribution patterns differed between calcified and non-calcified coronary plaques.

2. Materials and methods

2.1. Patient selection (Table 1)

The coronary arteries of 27 patients with renal disease and of 30 control patients (including 10 explanted hearts) were examined at the Department of Pathology, Erlangen-Nürnberg. Information on pre-existent coronary disease was not available. The study was elaborated and conducted according to the general guidelines for studies using human material. The study protocol was approved by the local Ethics Committee (University of Erlangen-Nürnberg, Germany, No. 2684). Information concerning coronary risk profile, e.g. hypertension, smoking, diabetes mellitus, as well as the laboratory findings, were obtained from hospital records. The mean laboratory values during last hospital stay were used for the statistical approach Table 1.

Renal (uremia) and non-renal control patients (control) were subdivided on histology according to the absence or presence of calcified coronary lesions into the following four groups:

(1) non-calcified control, (2) non-calcified uremia, (3) calcified control, and (4) calcified uremia.

2.2. Tissue preparation

At autopsy or heart explantation, samples of all three coronary arteries were obtained and analysed: ramus circumflexus of the left coronary artery (LCX), ramus interventricularis anterior of the left coronary artery (LAD), right coronary artery (RCA). As in a previous study [7] in each artery the segment with the most severe atherosclerotic lesion was selected for further histological examination and dissected into 2–3 mm thick slices. For morphological investigation, the specimens were fixed instantly after removal with formaldehyde (8%), embedded in paraffin, sectioned (4 μm) and stained using Haematoxylin-eosin (H&E), van Kossa technique (for demonstration of tissue calcification) and Elastica-van Gieson stain (for fibrous tissue).

On histology the lesions were classified according to Stary [33] from type II to type VII lesion: type II lesion corresponds to early lesion (fatty streak), type III to intermediate lesion with small extra cellular lipid pools, type IV to atheroma lesion with calcium granules in the lipid core region, type V to fibroatheroma lesion, type VI to complicated lesion with haematoma and/or thrombosis, and type VII to calcified lesion. The lesions were then regrouped as non-calcified (type II–VI) and calcified (type VII).

2.3. Immunohistochemical analysis

Immunolabeling was performed on paraffin sections using the following antibodies: mouse monoclonal antibody against CD40 (MCA1590, 1:250 Serotec, Düsseldorf, Germany), rabbit polyclonal 1 antibody against CD154 (C-20, 1:250, Santa Cruz Biotechnology, CA), rabbit polyclonal antibody