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Cadmium exposure as measured in blood in relation to macrophage density in symptomatic atherosclerotic plaques from human carotid artery

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ABSTRACT

Background and Aims: The general population is exposed to cadmium through diet and smoking. Cadmium is pro-atherogenic and pro-inflammatory in experimental and observational studies. Cadmium levels in blood and carotid plaque endarterectomies correlate. Cadmium concentrations are much higher in plaque-areas that most frequently rupture. Here we investigated if blood cadmium concentrations are associated with macrophage density and the accumulation of CD14 as indicator of macrophage activation by lipopolysaccharide (LPS) in endarterectomies from patients with symptomatic carotid plaques.
Methods: Endarterectomies from ninety nine patients were fixed in formalin, embedded in paraffin, serially sectioned and stained for assessment of morphology. As predefined, the two section levels with most prevalent plaque rupture were used for further analyses. Macrophages were assessed as area of staining for CD68 (%). Blood cadmium was measured with ICP-MS.
Results: The CD68 median [25,75 percentiles] from the average of both sections were higher in cadmium tertile 3 than in tertile 1 (9.8 [4.9,16.1] % and 3.8 (0.6,12.4) %, $p = 0.017$). This difference remained in a multiple linear regression analysis with ^{10}log meanCD68 as dependent variable and adjustment for sex, age, smoking, statin treatment, index event, time between event and surgery (beta coefficient 0.44 [95% CI 0.05–0.87]. CD14 was not associated with blood cadmium.
Conclusions: The results showed that blood cadmium was associated with proinflammatory macrophage density in the sections of carotid plaques with most frequent rupture, previously shown to contain most cadmium. No association between cadmium and LPS-mediated macrophage-activation was found. Cadmium exposure may promote plaque inflammation.

1. Introduction

Cadmium is a toxic metal that is found in rice, cereals, and root vegetables due to cadmium pollution of soil from fertilizers with high cadmium content, fall-out from industrial emissions and other urban sources [1]. Since tobacco smoke contains cadmium, smoking is also associated with high exposure to this metal. Hence, the general population is exposed to cadmium through diet and smoking leading to uptake via the intestines and the lungs [1]. The cadmium contents in urine and blood mirror long term cadmium exposure [1].

Cadmium exposure is not only associated with adverse effects on the kidneys, bone damage with fractures and certain types of cancer but seems also to have pro-atherogenic effects [2–9]. Cross-sectional and prospective studies have shown that the cadmium exposure of the general population is associated with cardiovascular death, myocardial infarction, stroke and peripheral artery

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disease [2–9]. In experimental studies cadmium exposure promotes the atherosclerotic process [10–12]. Disruption of the endothelium, increased apoptosis and oxidative stress promoting inflammation, have been suggested as underlying mechanisms [10,13]. Non-toxic cadmium-feeding to ApoE−/− mice induce atherosclerotic plaques including the occurrence of macrophages [11].

We have previously shown that cadmium is associated with the prevalence and size of atherosclerotic plaques in the carotid arteries in middle-aged women and men [14,15]. Since the prevalence of carotid atherosclerotic plaques increases with age and most plaques are asymptomatic, a critical issue is the process when a plaque turns into a complicated lesion with rupture and thrombosis, causing diseases such as ischemic stroke or myocardial infarction [16,17]. Inflammation is a very important feature of this transformation to a complicated lesion and macrophages play a key role [16–22].

In an attempt to clarify the role of cadmium in plaque tissue, we have used the well-known fact that symptomatic carotid plaques usually rupture upstreams to the maximum stenosis of the lesion where shear stress and inflammation are most pronounced [18–21]. In a recently published study we report that the concentrations of cadmium in blood and carotid plaque endarterectomies correlate, that the concentration is 50-fold higher in plaques than in blood, and that the cadmium level is around twice as high in the part of the plaques that most often ruptures [22].

The density of macrophages in carotid endarterectomies is associated with several known factors. First, stroke and transitory ischemic attacks (TIA) have a more inflammatory plaque phenotype than that linked to amaurosis fugax [23]. Second, surgery is performed with varying duration after the clinical events and it is important to realize that the macrophage content in these excised lesions may be more related to remodeling over time than to the original lesion causing a clinical event [24]. However, a potential proinflammatory effect of cadmium exposure should still be operative and visible in this phase of the atherosclerotic process.

In patients with acute symptomatic carotid artery stenosis, PET-CT examinations have shown that high serum LDL and low serum HDL cholesterol levels, respectively, are associated with inflammatory (18)fluorodeoxyglucose uptake in plaque tissue [25]. Therefore these serum lipids may affect a relation between cadmium and inflammation in plaques. Finally, the plaque stabilizing effect of statin treatment is a possible confounding factor to consider, although several observational studies have failed to show that macrophage accumulation in carotid plaques is decreased in statin-treated patients undergoing endarterectomy [26–29].

It is also important to examine if cadmium exposure affects the activation of proinflammatory macrophages. Activation of macrophages occurs typically by bacterial lipopolysaccharide (LPS) exposure. Then LPS is recognized by the innate immune system and leads to increased expression of the membrane-anchored receptor CD14 on macrophages which will trigger the inflammatory response [30–32]. CD14 is also expressed in soluble forms on monocytes/macrophages and predict future cardiovascular disease [33]. Previously, a study based on the present bio-bank has shown that 1) expression of CD14 in human monocyte-derived macrophages was increased by exposure to lipopolysaccharide; 2) the extent of CD14 staining was higher in the most severe lesion carotid plaque phenotypes [30]. There is also a possible link between cadmium and LPS, as previous studies have found an interaction between cadmium, LPS and metabolic syndrome and that cadmium exposure impairs the gut barrier, tentatively increasing the uptake of bacterial LPS [34,35].

We hypothesized that cadmium exposure is associated with inflammation in plaque tissue and contributes to complicated atherosclerotic lesions. Accordingly, the primary aim was to examine if cadmium concentration in circulating blood was associated with macrophage accumulation in the parts of symptomatic carotid plaque with highest prevalence of plaque rupture. A secondary aim was to examine if blood cadmium levels was associated with LPS activation of macrophages. In addition we explored whether blood cadmium levels were negatively associated with the plaque content of collagen and smooth muscle cells as measures of general plaque vulnerability.

2. Material and methods

2.1. Patients and carotid endarterectomies

The carotid atherosclerotic plaques were obtained from the Göteborg Atheroma Study Group biobank of patients who underwent carotid endarterectomy at the Sahlgrenska University Hospital (Gothenburg, Sweden) during 2003–2008 [21,22,30]. Criteria qualifying for surgery were minor ischemic stroke, transient ischemic attack (TIA) or amaurosis fugax and a high-grade carotid stenosis (≥70% determined with the European Carotid Surgery Trial method). Clinical information was obtained through questionnaires and medical records. Venous blood samples were drawn and stored in −80 °C.

In this bio-bank there were 99 serially sectioned endarterectomies (Fig. 1) which had been fixed in formalin and embedded in paraffin and with available classification of plaque severity and immunohistochemical assessment of macrophage content (see below). These patients were included in the study with the aim to select the two section levels with the highest prevalence of plaque rupture for further analyses.

The selected patients (n = 99) were compared with the remaining patients in the biobank who had no serially sectioned endarterectomies (n = 193): age (mean 69.3 versus 70.7 years, p = 0.16), sex (female sex 26 versus 31%, p = 0.39), current smoking (34 versus 36%, p = 0.81), statin treatment (72 versus 71%, p = 0.77). Neither were there any significant differences in the distribution in index events (data not shown, p = 0.32).

The study was carried out in accordance with the Declaration of

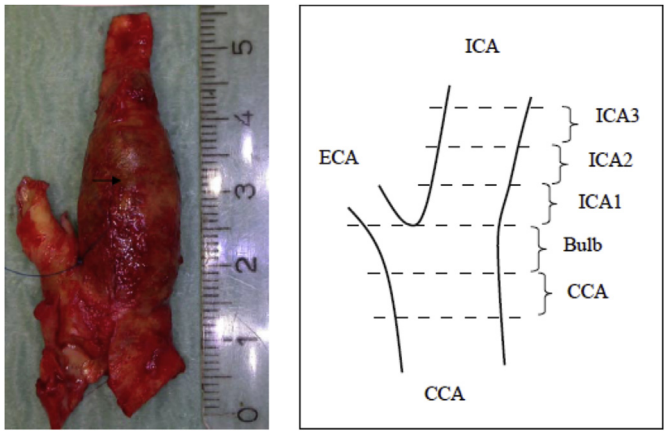

Fig. 1. Description of a highly stenotic carotid plaque and the division of the endarterectomies. The figure shows to the left a plaque after endarterectomy and to the right a drawing of the common carotid artery (CCA) that divides into to the internal (ICA) and external (ECA) arteries. The flow divider in the bifurcation between ICA and ECA, is easily recognized in most endarterectomies and was used as the zero point when the endarterectomies were divided into 3 mm thick blocks and named as is shown. Sections were taken on the side facing blood flow. The bulb and ICA1 blocks were used in the further analyses as plaque rupture was most frequent here.