Environmental Research 152 (2017) 185–191

Contents lists available at ScienceDirect

Environmental Research

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

Cadmium exposure is associated with soluble urokinase plasminogen activator receptor, a circulating marker of inflammation and future cardiovascular disease

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ARTICLE INFO

Keywords:
Cadmium
Biomarker
Inflammation
Soluble urokinase-type plasminogen activator receptor
Neutrophil to lymphocyte ratio

ABSTRACT

Background: Diet and smoking are the main sources of cadmium exposure in the general population. Cadmium increases the risk of cardiovascular diseases, and experimental studies show that it induces inflammation. Blood cadmium levels are associated with macrophages in human atherosclerotic plaques. Soluble urokinase-type plasminogen activator receptor (suPAR) is an emerging biomarker for cardiovascular events related to inflammation and atherosclerotic plaques. The aim was to examine whether blood cadmium levels are associated with circulating suPAR and other markers of inflammation.

Methods: A population sample of 4648 Swedish middle-aged women and men was examined cross-sectionally in 1991–1994. Plasma suPAR was assessed by ELISA, leukocytes were measured by standard methods, and blood cadmium was analysed by inductively coupled plasma mass spectrometry. Prevalent cardiovascular disease, ultrasound-assessed carotid plaque occurrence, and several possible confounding factors were recorded.

Results: After full adjustment for risk factors and confounding variables, a 3-fold increase in blood cadmium was associated with an 10.9% increase in suPAR concentration ($p < 0.001$). In never-smokers, a 3-fold increase in blood cadmium was associated with a 3.7% increase in suPAR concentration ($p < 0.01$) after full adjustment. Blood cadmium was not associated with C-reactive protein, white blood cell count and Lp-PLA2 but with neutrophil/lymphocyte ratio in one of two statistical models.

Conclusions: Exposure to cadmium was associated with increased plasma suPAR in the general population, independently of smoking and cardiovascular disease. These results imply that cadmium is a possible cause for raised levels of this inflammatory marker.

1. Introduction

The general population is exposed to cadmium by consuming food items such as rice, wheat, vegetables, and potatoes; and, for those who smoke, by uptake of cadmium from tobacco smoke in the lungs (Nordberg et al., 2015). Cadmium is mainly accumulated in the kidneys, and is eliminated very slowly, with a half time of decades. Cadmium in blood is affected by recent exposure, but at stable environmental exposure it reflects the body burden (Nordberg et al., 2015; Akerstrom et al., 2013). Cadmium is a non-essential metal with toxic effects resulting in increased risks of adverse effects on kidney and bone as well as some malignant diseases and premature death

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; CRP, C-reactive protein; HDL, high density lipoprotein; IQR, interquartile range; LDL, low density lipoprotein; Lp-PLA2, Lipoprotein-associated phospholipase A2; MDCS, Malmö Diet and Cancer Study; NHANES, National Health and Nutrition Examination Survey; SD, standard deviation; suPAR, soluble urokinase-type plasminogen activator receptor; uPAR, urokinase-type plasminogen activator receptor; WBC, white blood cell count

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http://dx.doi.org/10.1016/j.envres.2016.10.019
Received 14 July 2016; Received in revised form 26 September 2016; Accepted 19 October 2016

(Åkesson et al., 2014; Larsson et al., 2015). An accumulating body of data shows that cadmium exposure is also associated with increased risk of atherosclerotic diseases such as myocardial infarction and stroke (Larsson et al., 2015). The evidence is based on experimental (Subramanyam et al., 1992; Messner et al., 2009; Knoflach et al., 2011; Almenara et al., 2013), cross-sectional, and prospective studies of clinical events (Tellez-Plaza et al., 2013; Barregard et al., 2016), and on investigations of subclinical atherosclerosis in different vascular beds (Fagerberg et al., 2012, 2015; Bergström et al., 2015).

There is an uptake of cadmium in many organs including liver, arterial vessel wall, and atherosclerotic plaques (Bergström et al., 2015; Vuori et al., 1979). Many toxic mechanisms have been demonstrated, with the pro-inflammatory effects particularly in focus (Olszowski et al., 2012). The majority of these studies, which have been performed *in vitro* and *in vivo* in different cell lines, in animal models, and in humans, suggest that cadmium causes up-regulation of the mediators and markers of inflammation (Olszowski et al., 2012).

Inflammation is an important component of the atherosclerotic disease process (Packard and Libby, 2008). Many circulating inflammatory biomarkers have been linked to cardiovascular diseases, although the underlying mechanisms are poorly known (Lubrano et al., 2015). Serum C-reactive protein (CRP) and white blood cell count (WBC) are well-known such markers (Emerging Risk Factors Collaboration, Kaptoge et al., 2010; Madjid et al., 2004). The ratio of the neutrophil to lymphocyte count may carry specific and strong information on future coronary risk (Fowler et al., 2013). Lipoprotein-associated phospholipase A2 (Lp-PLA2), which is involved in the metabolism of oxidized low-density lipoprotein (LDL), is an established predictor of future cardiovascular disease (Maiolino et al., 2015).

The urokinase-type plasminogen activator receptor (uPAR) is a membrane-bound protein that is highly expressed in macrophages and in symptomatic atherosclerotic plaques, and is locally enriched within symptomatic plaques (Svensson et al., 2004, 2008; Steins et al., 2004). Both uPAR and cadmium seem to accumulate in close connection to macrophages in symptomatic carotid plaques (Svensson et al., 2008; Fagerberg et al., 2016). uPAR is associated with fibrinolysis, cell migration, and matrix degeneration (Hodges et al., 2015). It is also released from the cell surface into blood in a soluble form (suPAR) and may reflect a pathophysiological pathway more closely linked with subclinical organ damage than CRP, which to a large extent mirrors obesity and dysmetabolism (Hodges et al., 2015). suPAR levels reflect low-grade inflammation, and are associated with lifestyle factors such as smoking, alcohol intake, and sedentary lifestyle; in addition, women have higher levels than men (Hodges et al., 2015). suPAR is a novel circulating biomarker, and may outperform traditional markers of inflammation such as CRP in prognosticating different cardiovascular diseases (Hodges et al., 2015; Persson et al., 2012, 2014). No previous studies have examined whether cadmium exposure is associated with suPAR.

Two population-based studies from National Health and Nutrition Examination Survey (NHANES) have shown associations between urinary cadmium levels and high-sensitivity CRP levels, and these associations also hold after adjustment for smoking (Lin et al., 2009; Colacino et al., 2014) and in never-smokers (Colacino et al., 2014). However, one of these studies found a negative association between cadmium and WBC (Colacino et al., 2014). In contrast, a previous *in vivo* study on rats demonstrated a potential positive association between acute cadmium exposure and increased WBC (Morgan et al., 1984).

It is known that food items such as fibre and vegetables have the dual effects of both being important contributors to a high cadmium intake and conferring an anti-inflammatory effect (Nordberg et al., 2015). This anti-inflammatory effect was suggested in the present cohort, in which a high intake of fibre, fruits, and vegetables was significantly correlated with lower CRP levels (Dias et al., 2015). These data suggest that diet composition may reduce the pro-inflammatory effect of cadmium exposure; and, indeed, in NHANES a diet rich in anti-inflammatory nutrients was associated with a reduction in CRP (Colacino et al., 2014).

We hypothesized that cadmium exposure is associated with an increase in circulating suPAR concentrations independently of known covariates related to sex, lifestyle, and anti-inflammatory nutrients. We also wanted to explore the associations between cadmium exposure, CRP, total WBC, the neutrophil to lymphocyte ratio, and Lp-PLA2.

2. Materials and methods

As previously described, all men and women in the city of Malmö, Sweden, born between 1923 and 1950, were invited to participate in the Malmö Diet and Cancer Study (MDCS), which aimed to examine the association between dietary factors and cancer (Berglund et al., 1993). Between 1991 and 1994, a random selection of half of those included in the MDCS and born between 1926 and 1945 were invited to a cardiovascular sub-study including ultrasound measurement of carotid atherosclerosis (Fagerberg et al., 2015; Hedblad et al., 2000). Of the 6103 participants in the cardiovascular sub-cohort fasting blood samples were provided by 5540. The present study included participants with data available on cadmium in blood and circulating biomarkers of inflammation (n=4648). Reasons for exclusions were missing data on cadmium in blood (n=592), suPAR (n=204) CRP (n=134) and Lp-PLA2 activity (n=136). Mean age was identical between the total sub-cohort and the participants in the present study (57.5 years in both cases). Women constituted 60.0% and 59.2%, respectively, of these cohorts.

2.1. Cardiovascular risk factors

As previously reported the participants visited the study centre on three occasions (Hedblad et al., 2000; Wirfält et al., 2001; Rosvall et al., 2005). At the first visit the study questionnaires were distributed and the anthropometric measurements were performed. Non-fasting blood samples were taken, haematocrit, lymphocytes and neutrophils were analysed. Erythocytes were separated and stored in biobank. At the second visit trained interviewers conducted a diet history interview and checked the correctness of questionnaires. At the third visit fasting blood samples were drawn and stored in a biobank (Supplement).

Dietary habits were assessed by using a modified diet-history method, combining a 168-item quantitative diet history questionnaire, a 7 d menu book and a 1 h dietary interview (Wirfält et al., 2001; Dias et al., 2015). Fibre and vegetable intake data were adjusted for total energy consumption (g/MJ). The diet data were in the regression analyses adjusted for season during year the examination was performed (see Supplement).

Participants were categorized into never-smokers, ex-smokers, and current smokers. Information on pack-years was available in 3707 subjects. (Fagerberg et al., 2015). Daily alcohol intake was calculated.

Definitions of cardiovascular risk factors in the present study has been presented previously (Manjer et al., al., 2002; Rosvall et al., 2000). Educational level was defined as the number of years of completed education. Low education included those who had < 9 years of education. A low leisure-time physical activity was defined as individuals belonging to the bottom quartile of the obtained physical activity score, modified from the Minnesota Leisure Time Activity Questionnaire. Alcohol intake was classified as high in women consuming > 30 g/day and in men with > 40 g/day. The self-administered questionnaire was used to acquire information on use of lipid-lowering, antihypertensive and antidiabetic drugs at baseline. Subjects were classified as having diabetes mellitus if they reported the diagnosis in the questionnaire, if they had a fasting venous whole blood glucose ≥6.1 mmol/L, or if they were taking medication for diabetes mellitus. Biochemical measurements of blood glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides, as well as the