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Co-exposure to lead increases the renal response to low levels of cadmium in metallurgy workers☆

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HIGHLIGHTS

- We performed a cross-sectional survey in 122 metallurgic refinery workers.
- We assessed the effect of co-exposure to Cd and Pb on early renal biomarkers.
- The exposure to Cd and Pb was low to moderately high, respectively.
- Pb increases the strength of the association between Cd and renal biomarkers.

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ABSTRACT

Purpose: Research on the effect of co-exposure to Cd and Pb on the kidney is scarce. The objective of the present study was to assess the effect of co-exposure to these metals on biomarkers of early renal effect.
Methods: Cd in blood (Cd-B), Cd in urine (Cd-U), Pb in blood (Pb-B) and urinary renal biomarkers, i.e., microalbumin (μ-Alb), beta-2-microglobulin (β_2-MG), retinol binding protein (RBP), N-acetyl-β-D-glucosaminidase (NAG), intestinal alkaline phosphatase (IAP) were measured in 122 metallurgic refinery workers examined in a cross-sectional survey.
Results and conclusions: The median Cd-B, Cd-U, Pb-B were: 0.8 μg/l (IQR = 0.5, 1.2), 0.5 μg/g creatinine (IQR = 0.3, 0.8) and 158.5 μg/l (IQR = 111.0, 219.3), respectively. The impact of Cd-B on the urinary excretion of NAG and IAP was only evident among workers with Pb-B concentrations ≥75th percentile. The association between Cd-U and the renal markers NAG and RBP was also evidenced when Pb-B ≥75th percentile. No statistically significant interaction terms were observed for the associations between Cd-B or Cd-U and the other renal markers under study (i.e., μ-Alb and β2-MG). Our findings indicate that Pb increases the impact of Cd exposure on early renal biomarkers.

1. Introduction

Cadmium (Cd) and lead (Pb) are widespread occupational and environmental toxicants. Inhalation and ingestion are the two main routes of exposure to Cd and Pb (Johri et al., 2010), inhalation being the primary route of occupational exposure. After absorption, Cd is transported in the blood by albumin to the liver where it bounds to metallothionein (MT). The Cd-MT complex is then released back into the circulation. This complex of low molecular weight is freely filtered through the glomerulus and reabsorbed by the proximal tubule (PT) (Johri et al., 2010; Nordberg, 2009; Sabath and Robles-Osorio, 2012). Cd up-regulates the MT production in the liver and kidney to limit the toxicity of unbound Cd. The kidney is the primary target of toxicity with respect to chronic exposure to Cd (Johri et al., 2010). When the renal capacity to produce MT is overwhelmed, renal tubular dysfunction may occur, as reflected by an increased urinary excretion of low-molecular-weight (LMW) proteins e.g., beta-2-microglobulin (β_2-MG) and retinol binding protein (RBP), and renal proximal tubular damage characterized by an excretion

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of urinary enzymes, e.g., N-acetyl-β-D-glucosaminidase (NAG) and urinary intestinal alkaline phosphatase (IAP) (Chaumont et al., 2011; Järup, 2002; Kido et al., 1995; Roels et al., 1999). The exact cellular processes underlying Cd nephrotoxicity are incompletely understood. Growing attention is going towards the oxidative status of cells and the production of reactive oxygen species (ROS), which is considered to play an import role in Cd-induced toxicity (Devi et al., 2007; Garçon et al., 2007; Hambach et al., 2013a; Johri et al., 2010; Thijssen et al., 2007; Wieloch et al., 2012). The renal handling of Pb is much less understood (Chaumont et al., 2012; Gonick, 2011; Sabath and Robles-Osorio, 2012). In the circulation, most Pb binds to proteins in erythrocytes and is distributed to soft tissues (e.g. kidneys) and bone, the latter being the main depot for this metal. Pb is bound to low molecular weight proteins (<1% of the total) and filtered freely through the glomerulus and reabsorbed by PT (Sabath and Robles-Osorio, 2012). According to Chaumont et al. (2012) it is likely that Pb is reabsorbed by the PT as a complex of low molecular weight, similar to the Cd-MT complex. In the PT cells, Pb can cause mitochondrial damage, production of ROS, intracellular depletion of gluthatione and apoptosis (Sabath and Robles-Osorio, 2012). Pb exposure affects the glomerular function (e.g., renal hyperfiltration) (de Burbure et al., 2006; Roels et al., 1994; Weaver et al., 2003), yet, renal tubular effects induced by Pb exposure are also reported by several authors (Chia et al., 1994; Gidlow, 2004; Navas-Acien et al., 2009; Staessen et al., 1992).

High exposure to Cd and Pb rarely occurs in most industrialized countries, but chronic low exposure to these metals is still a major public health issue (Wang and Fowler, 2008). Recent research suggests adverse renal effects in adults at low level of Cd and Pb exposure i.e., Cd in urine (Cd-U) < 2.0 μg/g creatinine and Pb in blood (Pb-B) < 10 μg/l (Ekong et al., 2006; Fadrowski et al., 2010; Fowler, 2009; Hambach et al., 2013a; Weaver et al., 2010, 2009). Furthermore, several epidemiological studies have underlined that isolated environmental or occupational exposure to Cd rarely occurs and is often associated with Pb exposure (Ekong et al., 2006; Johri et al., 2010; Roels et al., 1995; Wang and Fowler, 2008). However, as reviewed by Choudhury and Mudipalli (2008), research on the effect of combined exposure to Cd and Pb on the kidney in humans is scarce. Cd and Pb can interact with each other in a complex way (Johri et al., 2010). Following Wang and Fowler (2008), co-exposure to Cd and Pb may induce additive or synergistic interactions or even new effects that are not observed for single element exposure. Similarities in kidney target areas and mechanisms of toxicity (e.g., inhibition of sulfhydryl group containing enzymes and increased production of ROS) raise concerns regarding the possible nephrotoxicity of combined exposure to Cd and Pb (Navas-Acien et al., 2009).

The aims of this study were: (1) to measure indicators for Cd and Pb exposure in workers from a metallurgic refinery company; (2) to assess early biomarkers of subclinical renal effect (i.e., β2-MG, μ-Alb, RBP, NAG, IAP) in those workers; (3) to examine whether Pb exposure modifies (i.e., increases or decreases) the effect of Cd exposure on these biomarkers.

2. Material and methods

The study was designed as a cross-sectional survey. Data collection was performed by questionnaire and blood and urine were sampled. Approval of the study protocol was obtained from the Medical Ethics Committee of the University of Antwerp. All participants signed a written informed consent form.

2.1. Study population

A total of 122 male workers from a metallurgic refinery company in Belgium participated in this study. Selection criteria were blue collar workers, co-exposure to Cd and Pb (based on the occupational medical files), a seniority of minimum one year and voluntary participation. All subjects filled in a questionnaire on demographic characteristics, intake of medication, smoking habits, comorbidity (i.e., hypertension, diabetes, renal and/or urological problems) and working conditions.

2.2. Analytical methods

2.2.1. Collection of samples

Blood (venipuncture) and spot urine were collected for each subject ($n = 122$) during the annual occupational medicine examination. The samples were collected between January and December 2010 during the working hours between 9.00 a.m. and 4.00 p.m. They were immediately aliquoted and transferred to appropriate recipients, stored in a refrigerator (4–8 °C) and transported within a half day to the laboratory where they were stored at either 4 °C (Cd-B, Cd-U and Pb-B) or −80 °C (renal markers) till analysis. Samples that were too diluted (creatinine < 30 mg/dl) or too concentrated (creatinine > 300 mg/dl) were discarded (Barr et al., 2005; Lauwerys and Hoet, 1993; WHO, 1996).

2.2.2. Determination of cadmium and lead concentrations

Cd-B (whole blood), Cd-U and Pb-B (whole blood) were measured by electrothermal atomic absorption spectrometry with Zeeman background correction using a SIMAA 6000 apparatus (Perkin-Elmer) after a 1 + 4 dilution in a Triton X-100–HNO_3–$NH_4H_2PO_4$ matrix modifier solution. Concentrations were measured against an addition-calibration curve. Instrumental conditions were set according to methods published previously. The variability of the methods was <5% whilst detection limits in both blood and urine were 0.1 μg/l for Cd and 1 μg/l for Pb (D'Haese et al., 1991).

2.2.3. Determination of renal markers concentrations in urine

Urinary IAP (a brush border enzyme of the S3 tubular segment) was determined using an in-house developed enzyme-antigen immunoassay (EAIA) (detection limit: 0.02 U/l) (Verpooten et al., 1992) whilst for total NAG (a proximal tubule lysosomal enzyme) a colorimetric assay (Roche) (detection limit: 0.1 U/l) and for RBP (a marker of proximal tubule dysfunction) a latex immuno assay (detection limit: 5 μg/L) were applied (Bernard and Lauwerys, 1983). For β2-MG (a marker of proximal tubule dysfunction) measurement we used a particle-enhanced immunonephelometric method (Dade Behring) (detection limit: 0.02 mg/L) and an immunonephelometric method for the measurement of μ-Alb (a marker of glomerular damage) (Dade Behring) (detection limit: 2.4 mg/l). The urinary creatinine concentration was determined by means of the Jaffé method.

2.3. Statistical analysis

Samples below the levels of detection (LOD) were attributed a value of half LOD for statistical calculations (Glass and Gray, 2001). Continuous dependent variables were tested for normality by Kolmogorov–Smirnov testing. Concentrations of metals and renal markers are expressed as median and interquartile range (25th percentile, 75th percentile). Non-symmetrically distributed dependent variables are log-transformed. In order to operationalize the variable Pb-B, we first categorized Pb-B based on quartiles. After that we studied the associations between Cd-B/Cd-U and renal markers in the four quartiles of Pb-B (see also Section 3) in order to identify a threshold value for dichotomization. Then we operationalize Pb-B in an indicator variable that is equal to one for Pb-B concentrations ≥ threshold and zero for Pb-B concentrations < threshold. In order to explore the effect of Pb-B on the association between Cd (in blood and urine) and renal markers (i.e., modification or interaction), we performed a multiple linear regression analysis (adjusting for age and pack-years of smoking) including an interaction term Pb × Cd. We also considered the presence of modification by different parameters (i.e., ever smoking and analgesic/NSAID use) by including a second interaction term Pb × Cd × $\text{smoking}_{\text{ever}}$. All variables were entered simultaneously into the model. All tests were two-sided; p-values ≤ 0.05 were considered statistically significant; p-values between 0.05 and 0.10 were considered borderline statistically significant. Statistical analyses were performed using IBM SPSS for Windows (version 20).

3. Results

The characteristics of the study population are described in Table 1.

Table 2 presents the concentrations of Cd, Pb and renal markers. The median Cd-B and Cd-U were relatively low and below the Biological Exposure Index (BEI) of the American Conference of Governmental Industrial Hygienists (ACGIH), <5 μg/l and <5 μg/g creatinine, respectively (ACGIH, 2011). The median Pb-B concentration was moderately high, with 6.7% of the workers showing Pb-B concentrations above the 300 μg/l (BEI-ACGIH) (ACGIH, 2011). The normal values for β2-MG (<300 μg/g creatinine), μ-Alb (<20 mg/g creatinine) and NAG (<5.0 U/g creatinine) were exceeded in 3.3%, 4.1% and 1.6% of the samples, respectively, but none of the workers