Atherosclerosis 218 (2011) 207–213

Contents lists available at ScienceDirect

Atherosclerosis

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

Circulating levels of bisphenol A and phthalates are related to carotid atherosclerosis in the elderly

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ARTICLE INFO

Article history:
Received 18 February 2011
Received in revised form 18 April 2011
Accepted 2 May 2011
Available online 10 May 2011

Keywords:
Phthalate metabolites
Bisphenol A
Elderly
Serum levels
Atherosclerosis

ABSTRACT

Background and objective: Bisphenol A (BPA) levels have previously been associated with coronary heart disease (CHD). Since CHD is an atherosclerotic disease, we investigated if circulating levels of BPA and phthalate metabolites are related to atherosclerosis in a cross-sectional study.
Methods: In the population-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (1016 subjects all aged 70), the prevalence of overt plaques and echogenectity (grey scale median, GSM) of carotid artery plaques were recorded by ultrasound in both of the carotid arteries. The thickness (IMT) and echogenicity (IM-GSM) of the intima–media complex were also measured. Bisphenol A (BPA) and 10 phthalate metabolites were analyzed in serum by a API 4000 liquid chromatograph/tandem mass spectrometer.
Results: Mono-methyl phthalate (MMP) was related to carotid plaques in an inverted U-shaped manner. This pattern was significant after adjustment for gender, body mass index, blood glucose, blood pressure, HDL and LDL-cholesterol, serum triglycerides, smoking, antihypertensive treatment and statin use ($p = 0.004$). High levels of BPA, mono-isobutyl phthalate (MiBP) and MMP were associated with an echogenic IM-GSM and plaque GSM, while high levels of mono-2-ethylhexyl phthalate (MEHP) were associated with an echolucent IM-GSM and plaque GSM ($p < 0.0001$ after adjustment).
Conclusion: The phthalate metabolite MMP was related to atherosclerotic plaques in an inverted U-shaped manner independently of CV risk factors. Some phthalates and BPA were also related to the echogenicity of the plaques, suggesting a role for plaque-associated chemicals in atherosclerosis.

1. Introduction

Bisphenol A (BPA) and phthalates are man-made high volume chemicals that have been used as components in plastics for more than 100 years [1]. BPA is mainly used as the monomer for making polycarbonate plastic (PC) but also widely as a hardener in the production of other plastics, in epoxy resins lining food and beverage containers and also in cash receipts. Phthalates are derivatives of phthalic acid used primarily as plasticizers to make plastic products more flexible. Certain plastics may contain up to 40–50% phthalate by weight. Consumer products containing plastics include food packaging materials, children's toys and water pipes but phthalates are also used in PVC-containing medical devices such as bags for blood and in personal care products such as cosmetics [2]. Since both BPA and phthalates do not become a chemically bonded part of the plastic matrix they can migrate from the plastic product. This means that the compounds are omnipresent in the environment; subsequently they have been detected in the body fluids of almost all humans.

Once in the body, ingested BPA is mainly conjugated with glucuronic acid which is eliminated in the urine. A fraction of the absorbed BPA may also distribute to body storage site(s) such as adipose tissue, followed by a slow, low-level release of BPA into the bloodstream [3]. Metabolism and elimination of phthalates is more complex. The phthalates are metabolized to the corresponding monoester metabolites, which are eliminated in the urine as glucuronide conjugates or are further metabolized.

BPA and phthalates act as endocrine disrupting compounds (EDCs), i.e. compounds capable of causing dysfunction in hormonally regulated body systems. The phthalates function mainly as anti-androgens, while the main action attributed to BPA is oestrogen-like activity [4–6].

During recent years, elevated circulating levels of environmental contaminants have been found to associate with both a number of cardiovascular risk factors (CV risk factors) such as hypertension, obesity and diabetes, as well as to the metabolic syndrome [7–10]. Furthermore, elevated circulating levels of persistent organic pollutants have been found to associate with prevalent coronary heart

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0021-9150/$ – see front matter © 2011 Published by Elsevier Ireland Ltd.
doi:10.1016/j.atherosclerosis.2011.05.001

disease (CHD) [7,11–15]. Recently, urinary levels of BPA were also found to associate with CHD in the NHANES sample [16].

The hallmark of CHD is atherosclerosis. Coronary and carotid artery atherosclerosis often go hand in hand [17] since atherosclerosis is regarded as a global disease. In the case of myocardial infarction, an atherosclerotic plaque in the coronary arteries ruptures and gives birth to an occluding thrombus. Although it should be noted that the events triggering plaque rupture might not be the same as those leading to plaque progression, most, albeit not all, risk factors associated with CHD events have also been found to be associated with plaque formation/progression.

Since BPA levels have been associated with CHD, we hypothesized that subjects with high circulating levels of BPA and phthalates more often show carotid atherosclerotic plaques and increased intima–media thickness (IMT). Furthermore, since we recently found that the echogenicity (grey scale media, GSM) of the intima–media complex (IM-GSM) in the carotid artery, a possible marker of lipid infiltration in the vascular wall, is a powerful predictor of future CV death [18], we also investigated if BPA and phthalates levels were associated with IM-GSM and GSM in overt plaques. For these aims, we used the population-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study [19] in which we have data on atherosclerosis and circulating BPA and phthalates levels in almost 1000 subjects. We related circulating levels of BPA and phthalates vs. four different atherosclerosis outcomes evaluated in both carotid arteries; presence/absence of overt atherosclerotic plaques, echogenecity of plaques (plaque GSM), intima–media thickness of the common carotid artery (IMT) and echogenecity of the intima–media complex (IM-GSM).

Table 1
Basic characteristics and major cardiovascular risk factors.

n	1016
Females (%)	50.2
Height (cm)	169 (9.1)
Weight (kg)	77 (14)
Waist circumference (cm)	91 (12)
BMI (kg/m^2)	27.0 (4.3)
Waist/hip ratio	0.90 (0.075)
SBP (mmHg)	150 (23)
DBP (mmHg)	79 (10)
Serum cholesterol (mmol/l)	5.4 (1.0)
LDL-cholesterol (mmol/l)	3.3 (0.88)
HDL-cholesterol (mmol/l)	1.5 (0.42)
Serum triglycerides (mmol/l)	1.3 (0.60)
Fasting blood glucose (mmol/l)	5.3 (1.6)
Antihypertensive treatment (%)	31.5
Statin use (%)	14.7
Current smoking (%)	11
Bisphenol A	3.76 (2.02–6.52)
MEHP (di-2-ethylhexyl phthalate DEHP, CAS No. 117-81-7)	4.53 (2.04–15.5)
MEP (diethyl phthalate (DEP) CAS No. 84-66-2)	11.6 (7.2–17.5)
MiBP (di-isobutyl phthalate (DBP, DiBP) CAS No. 84-69-5 and di-n-butyl phthalate (DBP, DnBP) CAS No. 84-74-2)	13.5 (9.3–29.3)
MMP (dimethyl phthalate (DMP) CAS No. 131-11-3)	1.49 (0.8–3.1)

Means are given with SD in parenthesis. The serum concentrations (ng/mL) of the evaluated environmental contaminants are given as medians and interquartile range in parenthesis. The name and the CAS number of the associated phthalate parent compounds of the phthalate metabolites are given in parenthesis. SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; BPA: bisphenol A; MEHP: mono-2-ethylhexyl phthalate; MEP: mono-ethyl phthalate; MiBP: mono-isobutyl phthalate and MMP: mono-methyl phthalate.

2. Materials and methods

2.1. Subjects

Eligible to the study were all subjects aged 70 living in the community of Uppsala, Sweden. The subjects were randomly chosen from the register of community living. A total of 1016 subjects participated giving a participation rate of 50.1%. The study was approved by the Ethics Committee of the University of Uppsala.

All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. An arterial cannula was inserted in the brachial artery for blood sampling and later regional infusions of vasodilators. The participants were asked to answer a questionnaire about their medical history, smoking habits and regular medication.

Blood pressure was measured by a calibrated mercury sphygmomanometer in the non-cannulated arm to the nearest mmHg after at least 30 min of rest, and the average of three recordings was used. Lipid variables and fasting blood glucose were measured by standard laboratory techniques.Basic characteristics are given in Table 1.

Approximately 10% of the cohort reported a history of coronary heart disease, 4% reported stroke and 9% diabetes mellitus. Almost half the cohort reported some cardiovascular medication (45%), with antihypertensive medication being the most prevalent (32%). Fifteen percent reported use of statins, while insulin and oral antiglycemic drugs were reported in 2 and 6%, respectively [19].

2.2. Carotid artery ultrasound evaluation

The carotid artery was assessed by external B-mode ultrasound imaging (Acuson XP128 with a 10 MHz linear transducer, Acuson Mountain View, California, USA). The common carotid artery, the bulb and the internal carotid artery at both sides were visually investigated for the presence of plaque. A plaque was judged to be present in a particular carotid artery if a local thickening of the IMT was seen that was more than 50% thicker than the surrounding IMT in any part of the carotid artery investigated, and also if the atherosclerosis was extensive (IMT > 1.2 mm in all carotid segments) without focally thickened parts. We recorded whether carotid plaques were present in none, one or in both of the carotid arteries. Two readers evaluated the presence/absence of plaque in the carotid arteries. Their judgment on this matter was the same in >95% of the investigated arteries.

The intima–media thickness (IMT) was evaluated in the far wall in the common carotid artery 1–2 cm proximal to the bulb.

The images were digitised and imported into the AMS (Artery Measurement Software) automated software [20] for dedicated analysis of intima–media thickness and the grey scale median of the intima–media complex. A maximal 10 mm segment with good image quality was chosen for intima–media thickness-analysis from the carotid artery. The programme automatically identifies the borders of the intima–media thickness of the far wall and the inner diameter of the vessel and calculates intima–media thickness and the diameter from around 100 discrete measurements through the 10 mm long segment. This automated analysis could be manually corrected if not found appropriate at visual inspection. The given value for carotid artery intima–media thickness is the mean value from both sides.

A region of interest was placed manually around the intima–media segment that was evaluated for intima–media thickness and the programme then calculated the echogenicity in the intima–media complex from analysis of the individual pixels within the region of interest on a scale from 0 (black) to 256 (white). The blood was used as the reference for black and the adventitia was the reference for white. The GSM value given is the mean value from both sides.