Science of the Total Environment 442 (2013) 20–25

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

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

15-F_{2t} isoprostane as biomarker of oxidative stress induced by tobacco smoke and occupational exposure to formaldehyde in workers of plastic laminates

Valeria Romanazzi [a,*], Valentina Pirro [b], Valeria Bellisario [a], Giulio Mengozzi [c], Marco Peluso [d], Marco Pazzi [b], Massimiliano Bugiani [a], Giuseppe Verlato [e], Roberto Bono [a]

[a] Department of Public Health and Pediatric Sciences, University of Torino, Via Santena 5 bis, 10126 Torino, Italy
[b] Department of Chemistry, University of Torino, Via Pietro Giuria 5, 10126 Torino, Italy
[c] Clinical Chemistry Laboratory, San Giovanni Battista Hospital, C.so Bramante 88, 10126 Torino, Italy
[d] Cancer Risk Factor Branch, ISPO-Cancer Prevention and Research Institute, Via Cosimo il Vecchio 2, 50139 Firenze, Italy
[e] Unit of Epidemiology and Medical Statistics, University of Verona, Strada Le Grazie 8, 37134 Verona, Italy

HIGHLIGHTS

- Plastic laminate workers are exposed to air formaldehyde.
- Urinary 15F_{2t}-IsoP is an efficient biomarker of oxidative stress.
- Urinary cotinine is a sensitive and specific biomarker of tobacco smoke.
- Formaldehyde and tobacco smoke independently induce oxidative stress in humans.

ARTICLE INFO

Article history:
Received 21 August 2012
Received in revised form 10 October 2012
Accepted 12 October 2012
Available online 21 November 2012

Keywords:
15-F_{2t} isoprostane
Oxidative stress
Formaldehyde
Tobacco smoke

ABSTRACT

Background: Formaldehyde (FA) is a suspected human carcinogen capable of inducing oxidative stress through different metabolic ways. FA may originate from tobacco smoke, several environmental sources, as well as occupational sources, like furnishing industries specialized in the production of pressed-wood and laminate products.
Object: Our aim was to investigate the role of tobacco smoke and occupational exposure to air-FA in the induction of oxidative stress status by comparing FA-exposed with non-exposed subjects who smoked or did not.
Methods: Enrollment of 105 subjects was made in an industry of plastic laminates, including both workers directly exposed to FA and non-exposed office personnel, as control group. 15-F_{2t} isoprostane (15-F_{2t} IsoP), detected by ELISA technique and urinary cotinine, detected by GC-MS, were used for evaluating oxidative stress and tobacco smoke exposure, respectively. Air-FA levels were detected by GC-MS.
Results: FA concentrations were significantly higher in subjects occupationally exposed than the controls. Smoking habits and air-FA exposures independently induce the formation of 15-F_{2t} IsoP and increase the oxidative stress level.
Conclusions: Our findings show, for the first time, that 15-F_{2t} IsoP presents a dependency from both the smoking habit and air-FA exposures, and consequently, that these breathable pollutants could be considered as two important independent risk factors in increasing the oxidative stress in human beings.

1. Introduction

Oxidants, anti-oxidants and free radicals often play a useful role in cellular signaling, control of vascular tones, cell generation, and defense against microorganisms. Their formation is the result of an evolutive process. The oxidative balance can be disturbed by several adverse environmental and/or occupational conditions, causing an uncompensated increase of pro-oxidants (Basu, 2010). As a consequence, oxidative modification of cellular macromolecules, induction of cell death by apoptosis or necrosis, damage of structural tissue may occur (Lykkesfeldt, 2007). Cells living under aerobic conditions are continuously exposed to a large number of oxidizing compounds from several endogenous and exogenous sources. Urban air is a typical exogenous mixture of chemical compounds that can induce carcinogenic activity, oxidative stress and toxicity. For example, several pollutants (PM, PAHs, benzene, etc.), emitted from cars (Rusconi et al., 2011) may: (i) inhibit cell-mediated immunity toward infectious agents, (ii) exacerbate respiratory allergy, (iii) cause DNA damage

Abbreviation: FA, formaldehyde; air-FA, professional exposure to formaldehyde; ELISA, enzyme-linked immuno-sorbent assay; GC-MS, gas chromatography–mass spectrometry; IsoP, 15-F_{2t} isoprostane; crea, creatinine.

* Corresponding author. Tel.: +39 011 670 5818; fax: +39 011 236 5818.
E-mail address: valeria.romanazzi@unito.it (V. Romanazzi).

0048-9697/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2012.10.057

and (iv) induce lung cancer, after a long-term exposure (Rossner et al., 2008a,b). Many epidemiologists related the presence of an oxidative stress status in human beings with traffic emissions and air pollution (Rossner et al., 2011).

Formaldehyde (FA) is a breathable pollutant present in both living and working environments and is considered the prevalent carbonyl species in the urban atmosphere. FA is emitted by several primary sources (Bono et al., 2010a), it is formed in the troposphere by photochemical hydrocarbon-oxidation processes (Correa et al., 2003; Flyvholm and Andersen, 1993; Salthammer et al., 2010), and is also a component of tobacco smoke (Godish, 1989; Uchiyama et al., 2010). Production and use of FA in the manufacture of resins, paints, disinfectants, preservatives, and a variety of other chemicals or industrial products make this chemical compound potentially breathable in several (indoor) working and living environments (Kelly et al., 1999; Zhang et al., 2010b). Thus, FA is nowadays a relevant topic to be studied in environmental and occupational health studies (Nielsen and Wolkoff, 2010; Zhang et al., 2010b).

FA can induce local irritations, acute and chronic toxicity, and genotoxic and carcinogenic activity (IARC, 2006; Schmid and Speit, 2007; Speit et al., 2007), as confirmed by an increased incidence of nasopharyngeal cancer in some types of FA-exposed workers (Duhayon et al., 2008; Hauptmann et al., 2004), the relationship reported between FA and leukemia (Zhang et al., 2009, 2010a), and a significant positive association between FA exposure and childhood asthma (McGwin et al., 2010).

Rager et al. have recently suggested that FA alters the expression of 89 microRNA (miRNA) profiles targeting mRNAs linked to numerous biological pathways, including those involved in the inflammatory response (Rager et al., 2011). In some molecular epidemiology studies, oxidative properties of FA have been reported in rats and humans (Bono et al., 2010b). Different metabolic pathways, i.e., the production of FA detoxifying enzymes after FA exposure, appeared to be involved. (Im et al., 2006; Kum et al., 2007). F_2-isoprostanes (F_2-IsoPs) are prostaglandin-like bioactive compounds formed in vivo from the free radical-catalyzed peroxidation of essential fatty acids. F2-IsoPs are stable, robust molecules and are detectable in all human tissues and biological fluids analyzed, including plasma, urine, bronchoalveolar lavage fluid, cerebrospinal fluid, and bile. Based on their mechanism of formation, four F_2-IsoP regioisomers are generated. Compounds are denoted as 5-, 12-, 8-, or 15-series regioisomers depending on the carbon atom to which the side chain hydroxyl is attached; thus the compound under investigation belongs to the last regioisomer. Moreover, "*2t*" is for the *trans* position of the oriented side chain to the prostane ring in the 15-F_{2t}-IsoP (Roberts and Milne, 2009).

The metabolic fate of 15-F2t-IsoP in humans has been assessed in previous studies (Mitsumoto et al., 2008; Morrow et al., 1999; Roberts and Morrow, 2000). Findings of these studies show the usefulness of 15-F_{2t}-IsoP in assessing oxidant stress in vivo both in animal models and in humans.

In fact, they appear to be related to quite a number of human diseases, although a clear correlation between those pathological conditions and an oxidative stress status is far from being proven (Giustarini et al., 2009). Moreover, F_2-IsoPs seem to play a role in acute and sub-clinical chronic inflammations (Basu et al., 2009). Since F_2-IsoPs can be detected in urine samples, which sampling is possible with a non-invasive procedure, they have been proposed as a suitable biomarker for airways inflammation (Basu, 2008) and asthma diagnosis (Wedes et al., 2009), with the scientific community agreement.

Considering that FA and tobacco smoke have toxic and cancerogenic activities in different biological districts and may play a role in the onset of oxidative stress (Bono et al., 2010b; Campos et al., 2011), the potential of urinary 15-F_{2t} IsoP as indicator of oxidative stress induced by FA and tobacco smoke was investigated in this study, for the first time.

Healthy subjects, working in one of the world's leading manufacturers of decorative laminates located in the north-western part of Italy, were enrolled as volunteers. Workers exposed to FA and non-exposed office personnel, as control group, were statistically compared. To evaluate the FA exposure, air-FA was quantified, while tobacco exposure was measured by urinary cotinine, a metabolite of nicotine.

2. Material and methods

2.1. Epidemiological sample

51 healthy male workers of an industry of decorative laminates were recruited as subjects potentially exposed to FA. The decorative laminate sheeting is made of melamine and phenolic resins reacting with aldehydes during the thermosetting process. The resins are laminated onto layers of kraft paper topped with a decorative sheet. 54 other male subjects were enrolled as controls from some offices and laboratories of the National Health System, where FA was not used.

All the subjects involved in this study live and work in Bra (town located in Piedmont region, Italy, counting 28,000 inhabitants, 250 m a.s.l.) or in its immediate surroundings. Only males were selected for this epidemiological investigation, since gender is actually debated as confounding factor in the 15-F_{2t} IsoP formation, moreover only male workers are usually employed in plastic laminate industries. All subjects were informed about the objective of the study and gave a written informed consent, voluntarily.

All samplings were executed during summer. For each subject, air-FA samples were passively collected for an entire working shift (i.e., from 6 a.m. to 2 p.m.; about 8 h) in the middle of the working week (Wednesday). A spot of the first urine in the morning was also collected for urinary cotinine and 15-F_{2t} IsoP determinations.

2.2. Questionnaire

At the end of the working shift, a questionnaire was administered to each subject to acquire information about individual and clinical features (age, place of residence, hobbies, and therapies), smoking habits, profession (qualifications, seniority, and job-specific work), the presence and use of environmental and personal devices to prevent air exposure and health risks. More in detail, the description of smoking habits for all subjects was established a-priori. Both subjects who never smoked and smokers who had ceased smoking for at least 1 month were classified as "non-smokers", while subjects who smoked at least one cigarette per day were classified as "smokers".

2.3. Air-FA sampling and analysis

FA air sample was collected from each subject for the whole working shift (8 h) using a passive, personal air sampler working with radial symmetry (Radiello®), clipped near the breathing zone of the subject. Samplers were equipped with a specific sorbent tube containing 35–50 florisil mesh coated with 2,4-dinitrophenylhydrazine (DNPH). DNPH reacts with FA yielding 2,4-dinitrophenylhydrazone which was subsequently quantified by a GC-MS method, within the following 10 days. Cartridges were stored at −80 °C before GC-MS analysis. Each sample was eluted with 3 ml of toluene and shaken at room temperature for 15 min. An aliquot was transferred into a vial and then injected in a capillary Agilent Technologies 6890 gas chromatograph, interfaced to a 5973 MSD Inert Agilent single quadrupole mass spectrometer. A Gerstel CIS4 PTV injection system utilized an initial temperature of 65 °C followed by heating at 5 °C/s; with a final temperature of 320 °C, held for 10 min. The injection volume was 2 μl in splitless mode. The capillary column used was a HP-5MS 30 m × 0.25 mm × 0.25 μm film thickness. Initial column temperature was 70 °C, and increased at 20 °C/min up to 220 °C and at 30 °C/min up to 300 °C. The carrier gas