International Journal of Hygiene and Environmental Health 217 (2014) 287–293

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

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

Oxidative stress in adolescent passive smokers living in urban and rural environments

Roberto Bono[a,*], Valeria Bellisario[a], Valeria Romanazzi[a], Valentina Pirro[b], Pavilio Piccioni[c], Marco Pazzi[b], Massimiliano Bugiani[a], Marco Vincenti[b]

[a] University of Torino, Department of Public Health and Pediatrics, Torino, Italy
[b] University of Torino, Department of Chemistry, Torino, Italy
[c] Unit of Respiratory Medicine, National Health Service (ASL TO2), Turin, Italy

ARTICLE INFO

Article history:
Received 11 January 2013
Received in revised form 27 June 2013
Accepted 28 June 2013

Keywords:
Oxidative stress
15-F_{2t}-isoprostane
Passive tobacco smoke
Urban pollution
Adolescents

ABSTRACT

Purpose of this study was to study the oxidative stress status through the urinary 15-F_{2t}-isoprostane (15-F_{2t}-isoP) among a group of 168 adolescents, differently exposed to passive tobacco smoke. Subjects were enrolled, with written informed consent, between two populations of students living and attending school in two areas with different levels of urbanization in Piedmont Region, North-Western Italy. A general linear model (GLM) analysis was performed to evaluate the role of air pollution, dependent from selected degree of urbanization and of passive exposure to tobacco smoke, quantified through cotinine, in the synthesis of 15-F_{2t}-isoP, measured with ELISA technique.

Formaldehyde (FA) concentration in air was also evaluated as a primary confounding factor in oxidative stress but no significant differences between the two sites were found. Conversely, direct relationship between oxidative stress status and residence of adolescents was found: oxidative stress level was 31% higher for adolescents living in Chivasso (urban site) than for those living in Casalborgone (countryside area). Furthermore, also passive tobacco smoke exposure proved to play another important direct role in the distribution of 15-F_{2t}-isoP levels ($p < 0.0001$). Lastly, an inversely proportional relationship was found between the age of adolescents and 15-F_{2t}-isoP ($p < 0.0001$). Finally, the detection of such a sensitive biological response as a consequence of limited differences of environmental pollution and exposure to tobacco smoke passively breathed could provide new and useful knowledge for the appraisal of preventive strategies, particularly for young subjects.

Introduction

Indoor and outdoor air pollution, hazardous chemicals, noise, food and water contaminants are factors possibly associated with environment-related health outcomes, including respiratory diseases, allergies and asthma, cardiovascular diseases, neurological effects, reproductive and developmental disorders, and cancer. Among the environmental factors, urban outdoor air pollution, partly generated by car exhaust, and environmental tobacco smoke (Bono et al., 2005b) have become a problem of growing international interest (Bono et al., 2010a; Cohen et al., 2005; Tzivian, 2011).

Air pollution may present various physical, chemical, mutagenic and toxicological properties, according to geographical area and human socio-economic activities (Traversi et al., 2008, 2009). The resulting impact on human health may evolve with different characteristics and intensity levels (Bono et al., 2005a; Gomes et al., 2012; Plummer et al., 2012). Epidemiological studies performed in metropolitan areas revealed that the exposure to urban air pollution is a significant factor in the increasing prevalence of many diseases and mortality, even if its mechanisms of action remains partially unclear (Brauner et al., 2007; Brunekreef, 2007). In urban areas, prevalent contribution to air pollution arises from motor vehicle emissions (Gomes et al., 2012). Thus, many researchers focused their studies on exposure assessment and measurement of primary biological and/or adverse health effects on citizens exposed to traffic-related pollutants (Bind et al., 2012; Gan et al., 2012).

Environmental tobacco smoke (ETS) is a complex mixture of gases and particles comprising smoke produced by the cigarette while smoldering between puffs and smoke exhaled by the smoker during active smoking. ETS, involuntarily breathed in by non smokers, has been classified as carcinogenic to humans by International Agency for Research on Cancer (IARC, 2002) and by the National Toxicology Program of the US National Institutes of Health (NTP,

* Corresponding author at: Department of Public Health and Pediatrics, University of Torino, Via Santena 5 bis, 10126 Torino, Italy. Tel.: +39 011 670 5818; fax: +39 011 236 5818.
E-mail address: roberto.bono@unito.it (R. Bono).

1438-4639/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.ijheh.2013.06.008

2011). Due to exposure to ETS, the non active smokers also, including children, are at risk for smoking-associated health effects (Witschi et al., 1997a,b). Furthermore, the relationship between the smoking habit of adults and ETS exposure of their children have been recognized by many authors (Dept. Health & Human Serv., 2006; Dept. Health & Human Serv., 2007; Dostal et al., 2008) some of which have shown that 39–71% of children are currently exposed to ETS (Moshammer et al., 2006).

Cotinine, a metabolite of nicotine, is the biomarker more widely used for the specific assessment of ETS exposure (internal dose marker) during the last 20 h for smokers and during even longer periods in passive smokers, particularly children (Bono et al., 2005b). Thus, since exposure to tobacco smoke is constant over time, cotinine well represents the ETS daily intake.

Recently, many scientific evidences ascribe to air pollution, to vehicle exhaust emissions and ETS the effect to modulate the body's redox system through an increase of pro-oxidant species and a decrease of antioxidant molecules (Ghio et al., 2012; Henderson, 2008; Lobo Torres et al., 2012; Yang and Omaye, 2009; Zalata et al., 2007). This condition, defined as oxidative stress, is connected with several DNA lesions, including modifications of bases, which are considered potential causes of cancer (Ghio et al., 2012; Loeb, 2001). Moreover, the importance of oxidative stress in the respiratory health effects is partly attributed to airborne ultrafine particles which, with their large surface may prolong the effects of other inhaled pollutants (Li et al., 2003; Moller et al., 2002).

Oxidative stress can be induced by outdoor and indoor environments (residential, public or occupational) (Bono et al., 2010b). The indoor environments, particularly the sites where people smoke or have smoked, are often characterized by the highest levels of pollutants that induce oxidative stress (Fuselli et al., 2010). Accordingly, oxidative stress plays a crucial role in the inflammatory response to tobacco smoke (Doruk et al., 2011), frequently in co-exposure with airborne ultrafine particles (Mo et al., 2012). Tobacco smoke is a complex mixture of oxidizing compounds, capable to promote numerous biological damages, such as lipid peroxidation (Kalra et al., 1991; Morrow et al., 1995; Scherer, 2005), protein and thiol oxidation (Frei et al., 1991; Reznick et al., 1992), and oxidation of DNA (Park et al., 1998). The combustion-derived nanoparticles (CDNPs), widespread in the environment and in particular in the ETS, produce oxidative stress, inflammation and lung cancer. CDNPs can be redistributed to other organs, after pulmonary deposition (Donaldson et al., 2005). The knowledge of how exogenous and endogenous oxidants interact with molecules in the cells, tissues, and the epithelial lining fluid (ELF) of the lung is crucial for planning the most suitable prevention strategies.

F2-isoprostanes are specific products of lipid peroxidation and their metabolites were evaluated in vivo as potential biomarkers of oxidative stress status (Roberts and Morrow, 2000). F2-isoprostanes are a family of Prostaglandin (PG) F2α isomers, described as products of non-cycloxygenase after oxidative modifications of arachidonic acid, that resulted from free-radical attack of cell membrane phospholipids or circulating low density lipids (LDLs) (Lynch et al., 1994; Morrow et al., 1990). Thus, F2-isoprostanes, a chemically stable group of bioactive compounds, appear to play a role in acute and sub-clinical chronic inflammations (Basu et al., 2009) and are utilized as non-invasive markers of airways inflammation (Basu, 2008) and asthma (Wedes et al., 2009). They may describe the possible role of some exogenous factors in the expression of oxidative stress in selected populations. They can also be implicated in a large number of human diseases, even if a clear correlation between disease and oxidative stress is far from being proven for most pathological conditions (Giustarini et al., 2009).

In the fully aware that oxidative stress originates from many endogenous and exogenous factors, purpose of this study was to clarify the role in the biosynthesis of 15-F_{2t}-isoprostane (15-F_{2t}-IsoP) of two of these exogenous factors: exposure to tobacco smoke and air pollution. Given the scarcity of information in this regard, the study was conducted in a population of adolescents living in two areas of the Piedmont Region (North-Western Italy) characterized by different geographical conditions and levels of urbanization and taking into account their passive exposure to tobacco smoke.

Methods

Sampling sites

Two sampling sites were selected taking into account different urbanization, anthropization and vehicular traffic conditions. Chivasso is an urbanized town with about 26,000 inhabitants (514 inhabitants/km^2), located at 180 m a.s.l. close to Torino, the capital of Piedmont Region (900,000 inhabitants). Casalborgone (200 m a.s.l.) is a rural site, 12 km away from Chivasso, and populated by 1850 inhabitants (92 inhabitants/km^2). (Sources by Piedmont Region, 2011) (Fig. 1).

Epidemiological sample

All adolescents (N = 168) involved in the present study were volunteers who attended the same school district but in two different campuses, one in Chivasso (urban site) (N = 110) and one in Casalborgone (rural site) (N = 58), respectively. No other selection criteria was adopted. Since the subjects were underage, during a public meeting, parents and teachers were informed on the objective of this study; secondly a written informed consent was signed and delivered by each the participants' parents. Thus, the participation of all the human subjects did not occur until after informed consent was obtained. Sampling was carried out from March to April 2011, involving one class per day, on Wednesday or Thursday, according to a pre-established timetable. From each student, the following items were collected: (1) a questionnaire, gathering information about general features, (2) a urine sample for the quantification of urinary cotinine, 15-F_{2t} IsoP and creatinine (CREA), (3) spirometry data to evaluate respiratory health and vital capacity.

Questionnaire

One interviewer administered, during the school time, a questionnaire to each subject obtaining information on individual and clinical features, such as age, gender, residence, diet (dinner the day before), hobbies, therapies, and parent'smoking habits (as n. of cigarettes/day). The questionnaire used was mainly a synthesis of the most extensive questionnaire "SIDRIA", described in detail elsewhere (Migliore et al., 2005, 2009).

Urinary cotinine

Urinary cotinine was measured in order to consider the possible role played by passive tobacco smoke in the onset of an oxidative stress status. An aliquot of fresh urine was collected in the early morning and approximately at the same time from each volunteers, and stored at −80 °C prior to analysis, performed within 20 working days. 10 ml of urine was transferred into a glass tube and 4 g of NaCl, 500 μl of NaOH (5 M) and 10 μl of cotinine-d_3 (internal standard) were added. Subsequently, for two times, 2 ml of trichloromethane ($CHCl_3$) were added to the sample to perform liquid-liquid extraction which was carried out in a shaking wheel for 15 min. Sample was then centrifuged for 10 min at 1000 × g and the resulting organic phase was collected in a new glass tube and evaporated to dryness in a rotary evaporator at room temperature.