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Occupational exposure to formaldehyde and biological monitoring of Research Institute workers

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Abstract

Aim: The aim of this study was to verify the presence of a relationship between formaldehyde exposure in the work environment with biological markers of exposure and of effect. *Methods*: Exposure to formaldehyde (FA) of 36 workers in different laboratories of a Cancer Research Institute and biomarkers of exposure, such as formaldehyde human serum albumin conjugate (FA-HSA) and biomarkers of effect, such as chromosome aberration (CA), micronuclei (MN) and sister chromatid exchanges (SCEs) were measured in peripheral blood lymphocytes of the same workers. *Results*: Individual FA levels of exposure ranged from 4.9 μg/m^3 to 268.7 μg/m^3. Subjects with high FA exposure showed a significant increase of the biomarker of exposure FA-HSA, but biomarkers of effect did not show any significant differences. *Conclusions*: A significant relationship was observed between occupational exposure to FA and a biological marker of exposure (FA-HSA). The markers of effect used (CA, MN and SCE) failed to indicate the presence of genetic damage.

Keywords: Biological markers; Formaldehyde; Laboratory personnel; Occupational exposure; Chromosome aberration; Micronuclei; Sister chromatid exchanges; Human serum albumin conjugate; Smoking

1. Introduction

Formaldehyde (FA), also known as formalin, formal, and methyl aldehydes, is a colorless, flammable, strong-smelling gas. Outdoor exposure to FA can originate from many sources such as incinerators, photochemical smog, and jet engine exhaust [1–3]. Indoor exposure of relevant importance is found in hospitals and scientific institutions where FA is used as a sterilizing and preserving agent [4–7], and in places like schools, kindergartens, and mobile homes or apartments where there may be uncontrolled emissions of FA from tobacco smoking, building materials, or furniture [8–11].

Occupational exposure to FA occurs in a wide variety of occupations and industries [12–19]. The inhalation of vapors can produce irritation to the eyes, nose, and upper respiratory system. Whilst occupational exposure to high FA concentrations may result in respiratory irritation and asthmatic reactions, it may also aggravate pre-existing asthma [20]. Skin reactions, after exposure to FA, are very common because the chemical can be both irritating and allergenic [21,22].

In 1980, laboratory studies showed that exposure to FA could cause nasal cancer in rats [23]. These findings raised the question of whether FA exposure could also cause cancer

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in humans. Infact, in 1987, the U.S. Environmental Protection Agency (EPA) classified FA as a probable human carcinogen under the conditions of unusually high or prolonged exposure [24]. In 1995, the International Agency for Research on Cancer (IARC) concluded that FA is a probable human carcinogen [25]. Furthermore, the most recent evaluation by the IARC classified FA as a known human carcinogen (Group 1) [26–28], concluding that there is strong evidence that FA causes nasopharyngeal cancer, strong but not sufficient evidence for leukemia and limited evidence for sinonasal cancer.

Some studies showed that FA binds human serum albumin (HSA) covalently and found that subjects with multiple health complaints, who had been exposed chronically to FA, developed antiFA-HSA antibodies [29–30]. This observation suggests that the humoral immune response to the conjugate FA-HSA could provide a biological marker of FA exposure.

FA is slightly genotoxic in *in vitro* models, animals and humans, and increased numbers of DNA–protein cross-links have been found in the upper respiratory tract of monkeys and rats. Contradictory results were obtained *in vivo* in cytogenetic analysis of peripheral blood lymphocytes of exposed workers. The genotoxic effects of FA have been studied and cytogenetic effects, such as increased chromosomal aberrations, sister chromatid exchanges, and micronucleated cells, have been described [31–39].

In this study, we intend to verify the relationship between FA exposure and some biological markers – formaldehyde human serum albumin conjugate (FA-HSA), chromosome aberrations (CA), micronuclei (MN), sister chromatid exchanges (SCE) – in subjects who have been exposed to high concentrations of FA, in comparison to a reduced exposure group.

2. Materials and methods

The study was conducted on 36 workers from a Cancer Research Institute working in different departments: Anatomy and Pathology (7), Experimental Oncology (8), Toxicological Pharmacology (1), Data Elaboration Centre (6), and Environmental Epidemiology (14). Twelve workers were male and 24 were female. The mean age was 40.14 years (ranging from 27 to 52 years old). As for their smoking habits, 6 (16.7%) were active light smokers and 30 (83.3%) non-smokers.

A questionnaire was administered to each subject. The questionnaire contained demographic data, history of occupations and exposure, also in the domestic environment (i.e. paint), medical history, smoking habits, recent illnesses and medical treatment. The questionnaire was adapted from a validated questionnaire of the CREST (Cancer Respiratory Tract) Biological Bank of the Molecular Epidemiology Unit of the National Cancer Institute, Genoa, Italy. A diffusive sampler was clipped to the collar of the selected workers in order to measure the concentration of FA during a typical working day (8 h). A blood sample was then taken from each of the subjects at exactly the same hour at the end of the day in order to evaluate the biological markers.

2.1. FA analysis

FA was measured by means of diffusive radial samplers, containing 900 mg of 35–50 mesh florisil coated with 2,4-dinitrophenylhydrazine (DNPH) (RADIELLO by Maugeri Foundation, Environmental Research Centre–Padua, Italy). FA diffusing across the diffusive surface reacts with the DNPH on chemioadsorbing cartridge to form the corresponding dinitrophenylhydrazones. DNPH derivatives were extracted by CH_3CN (lichrosolv) and analyzed by HPLC equipped with an UV detector. A 150 mm (i.d. 4.6 mm) RP-C18 column was used and kept at 25 °C. A solvent mix, CH_3OH (lichrosolv Merck) 57% and H_2O (Milli Q $\geq$ 18 MΩ cm) 43% (v/v), was used. After 5 min a gradient was started: CH_3OH 60.6% for 8 min, then 87% for 10 min and to 57% for 5 min and an equilibration time of 5 min before a new analysis. The calibration curve for FA was obtained with a standard solution of the corresponding 2,4-dinitrophenylhydrazones, certified by RADIELLO.

2.2. FA-HSA

The Displacement Assay and the Enzyme-Linked ImmunoSorbent Assay were used. The Displacement Assay is a test based on the competitive reaction of human IgG antibody against FA-HSA with rabbit IgG antibody against the same molecular conjugate. Rabbit IgG showed a significant cross-reaction with FA-HSA allowing the Displacement Assay for the determination of human antibodies against FA-HSA conjugate. HSA and FA-HSA were adsorbed overnight to microplate wells and then were postcoated with bovine serum albumin. Human serum was incubated with antigens and, after washing, rabbit IgG was added. The reliability of immunological response to FA-HSA was evaluated by comparison to the response against HSA. Positive responses in human sera were revealed by a decrease of optical density (OD) compared to the optical density obtained with rabbit IgG (positive control). The subject response was considered positive when the sample optical density was lower than the positive control. Negative response resulted when the sample OD was greater or equal to that of the positive control. Optical densities were measured imposing a wavelength of 492 nm.

$$\text{Marker of FA exposure} = (\text{OD positive control}) - (\text{OD sample}).$$