Malondialdehyde−Deoxyguanosine Adduct Formation in Workers of Pathology Wards: The Role of Air Formaldehyde Exposure

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Formaldehyde is an ubiquitous pollutant to which humans are exposed. Pathologists can experience high formaldehyde exposure levels. Formaldehyde—among other properties—induce oxidative stress and free radicals, which react with DNA and lipids, leading to oxidative damage and lipid peroxidation, respectively. We measured the levels of air-formaldehyde exposure in a group of Italian pathologists and controls. We analyzed the effect of formaldehyde exposure on leukocyte malondialdehyde−deoxyguanosine adducts (M_1-dG), a biomarker of oxidative stress and lipid peroxidation. We studied the relationship between air-formaldehyde and M_1-dG adducts. Air-formaldehyde levels were measured by personal air samplers. M_1-dG adducts were analyzed by a ^{32}P-postlabeling assay. Reduction room pathologists were significantly exposed to air-formaldehyde with respect to controls and to the pathologists working in other laboratory areas ($p < 0.001$). A significant difference for M_1-dG adducts between exposed pathologists and controls was found ($p = 0.045$). The effect becomes stronger when the evaluation of air-formaldehyde exposure was based on personal samplers ($p = 0.018$). Increased M_1dG adduct levels were only found in individuals exposed to air-formaldehyde concentrations higher than 66 μg/m^3. When the exposed workers and controls were subgrouped according to smoking, M_1-dG tended to increase in all of the subjects, but a significant association between M_1-dG and air-formaldehyde was only found in nonsmokers ($p = 0.009$). Air-formaldehyde played a role positive but not significant ($r = 0.355$, $p = 0.075$, Pearson correlation) in the formation of M_1-dG, only in nonsmokers. Working in the reduction rooms and exposure to air-formaldehyde concentrations higher than 66 μg/m^3 are associated with increased levels of M_1-dG adducts.

Introduction

Formaldehyde (FA)[1] is an ubiquitous pollutant to which humans are chronically exposed via different routes. This chemical is also present in various forms of life, including humans (*1*). Commercially, it is widely employed in the production of resins containing urea, phenol, melamine, and, in a smaller extent, their derivatives. Furthermore, the high chemical reactivity of FA is exploited for preservation and disinfection in pathology laboratories, as well as for antimicrobial agent in consumer and/or cosmetic products. Human exposure to FA in an occupational environment has been considered several times by the American Conference Governmental Industrial Hygienists (ACGIH), from 1946 until the actual ceiling limit value (TLV-C) of 0.3 ppm (0.370 mg/m^3).

The reactive capacity of FA, shown by direct contact to target tissues, can induce local irritations, acute and chronic toxicity, and genotoxic and carcinogenic activity (*2*−*4*), as confirmed by an increased incidence of nasopharyngeal cancer in industrial workers, embalmers, and pathologist (*5, 6*), by the relationship demonstrated between FA and leukemia in a recent meta analysis (*7*), and by a significant positive association between FA exposure and childhood asthma (*8*). Thus, the widespread use of FA in many working and life contexts represents a potential risk factor for human health when this pollutant is assumed by contact or is breathed. This last type of exposure is probably the principal and more important route of human exposure, in both life and work environments.

FA is mainly genotoxic for chromosome to bacteria (*9*−*11*) and mammalian cells in culture, including human cells (*4, 12, 13*). Some end points were analyzed in vitro and in epidemiological studies, including the formation of micronuclei (MN) and sister chromatid exchange (SCE) in peripheral blood lymphocytes.

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[1] Abbreviations: ACGIH, American Conference Governmental Industrial Hygienists; CT, calf thymus; DNPH, 2,4-dinitrophenylhydrazine; DPCs, DNA−protein cross-links; LOD, limit of detection; M_1dG, 3-(2-deoxy-β-D-erythro-pentafuranosyl)pyrimido[1,2-α]purin-10(3*H*)-one dG adduct; FA, formaldehyde; LPO, lipid peroxidation; MDA, malondialdehyde; MN, micronucleus; NN, normal nucleotides; RCLB, red cells lysis buffer; ROS, reactive oxygen species; RAL, relative adduct labeling; SDS, sodium dodecyl sulfate; SCE, sister chromatid exchange; WCLB, white cells lysis buffer.

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To assess the DNA damage in chromosomes of populations occupationally exposed to FA, SCE was used as a cytogenetic biomarker in pathology laboratory workers (*14*−*16*). FA can also induce the formation of DNA adducts. In particular, FA-induced DNA adducts can be single-strained type (intrastrand cross-link) or double-strained type (interstrand cross-links) or can involve a reactive group of a protein to form DNA−protein cross-links (DPCs) (*2, 17*−*19*). DPCs are the primary and principal form of FA-induced DNA damage, in which histones probably are involved (*19, 20*). The DPCs formation due to FA exposure is confirmed by the reduction of electrophoretical migration of DNA damage observed by means of a Comet test and demonstrated also by an in vitro study where irradiated cells exposed to increasing FA concentrations show a reduction in DNA migration (*4, 18*).

However, FA also can induce oxidative stress. Indeed, FA can increase the formation of reactive oxygen species (ROS) in many tissues, which can interact with DNA and lipids, leading to oxidative damage and lipid peroxidation (LPO), respectively (*21*−*23*). Malondialdehyde (MDA) is a natural product of LPO that can react with DNA to form exocyclic adducts, including the 3-(2-deoxy-β-D-erythro-pentafuranosyl)pyrimido[1,2-α]purin-10(3*H*)-one dG (M_1dG), which, if not repaired, can induce base pair mutations and cause frame-shift mutations in reiterated sequences (*24, 25*). Previous studies have also shown that the formation of M_1dG adducts could be associated with increased cancer risk and tumor progression (*26*−*28*). M_1dG adduct measurement is also considered to be a biomarker that reflects exposure to air pollutants capable of inducing oxidative stress and ROS (*29*). In addition, dose-related induction of MDA production in plasma and liver was demonstrated by some experiments carried out in vivo on rats exposed to FA (*21, 30*).

Therefore, in the present study, we measured the levels of personal air-FA exposure in a group of pathologists working in three Italian pathology wards and in a group of controls. Then, we analyzed the effect of air-FA exposure on the levels of leukocyte M_1dG adducts, a biomarker of oxidative stress and LPO. The relationship between air-FA exposure levels and M_1dG adducts was also evaluated. Our results could provide the first indications to evaluate the doses of FA to which the workers are exposed and a consequent biological response planning future preventive actions.

Materials and Methods

Epidemiological Sample. Forty-four workers, recruited in three pathology wards of the Piedmont region in Italy, were recruited as subjects potentially exposed to FA, who are referred as cases in this paper. Contemporaneously, 32 students and workers in scientific laboratories of University of Torino not professionally exposed to FA were recruited as controls. For each of 76 subjects, an air-FA sample was collected for an entire working shift (8 h); data on personal medical history, smoking habits, and drug intake were also collected through a questionnaire administered at the end of the working shift when a sample of venous blood was collected as well. The description of smoking status of all of the subjects was a priori established, classifying them as "nonsmokers", the never smokers and the former smokers who had ceased smoking for at least 1 month, and "smokers" who smoked at least one cigarette per day. All subjects were informed about the objective of the study, and they gave written, informed consent.

Personal Air-FA. FA air samples were collected for a whole working shift (8 h) on Wednesday using passive, personal air samplers working with radial symmetry (Radiello), clipped near the breathing zone of the subject. Samplers were equipped with a specific sorbent tube containing florisil 35−50 mesh coated with 2,4-dinitrophenylhydrazine (DNPH). DNPH was reacted with FA, and changes by derivatization to the specific 2,4-dinitrophenylhydrazone−FA were quantified with a HPLC Perkin-Elmer equipped with an UV detector regulated at 360 nm: NIOSH Method no. 2016 (*31*). The instrument was set according the following specifications: pump Perkin-Elmer series 200, detector UV−vis Perkin-Elmer LC 295, dilutor model 401 Gilson, automatic sampler Gilson model 231, HPLC column, and cartridge 10 m LiChro CART 250-4. The instrumental conditions were as follows: mobile phase, 45% acetonitrile and 55% water; flux in column, 1 mL/min; and injection volume, 20 μL. The estimate limit of detection (LOD) was 0.05 μg/mL. The chemical desorption was done as follows: elution with 10 mL of acetonitrile and sonication for 20 min. Two hundred microliters of DNPH solution and a drop of concentrated perchloric acid were added to 1 mL of bubbled sample to promote derivatization to dinitrophenylhydrazone. The reaction proceeded for at least 30 min at room temperature, and then, the samples were transferred in microvials (300 μL) for HPLC analysis. The calibration curve was prepared using a calibration standard (the specific 2,4 dinitrophenilhydrazone formed as above) provided by the sampler manufacturer, Radiello, and had a certificated concentration of 3.83 μg/mL, expressed as FA. The calibration curve was prepared with a range of concentration between 0.05 and 3 μg/mL. The standard solutions and the blank were treated as samples.

Questionnaire. The same day of the air and blood sampling, at the end of shift, a questionnaire was administered to each subject to acquire information concerning the individual and clinical features (age, sex, place of residence, hobbies, and therapies), smoking habits, profession (qualifications, seniority, and job-specific work), and the presence and use of environmental and personal devices to prevent air exposure and health risks.

Blood Sampling, DNA Extraction, and Purification. For each subject, 10 mL of venous blood was collected at the end of the working shift in Vacutainer tubes containing Na-EDTA as an anticoagulant. Blood tubes were then kept in the dark at −20 °C and processed within few days. DNA was extracted from whole blood using a salting out method (*32*) to measure M_1dG adducts. Briefly, 1 mL of whole blood was washed with RCLB buffer (red cells lysis buffer: 10 mM TRIS, 5 mM $MgCl_2$, and 10 mM NaCl) to remove erythrocytes and then washed again with WCLB buffer (white cells lysis buffer: 10 mM TRIS, 10 mM EDTA, and 50 mM NaCl) to lyse leukocytes; furthermore, sodium dodecyl sulfate (SDS) for breaking cytoplasmic membrane and proteinase-K for histonic proteins digestion were added.

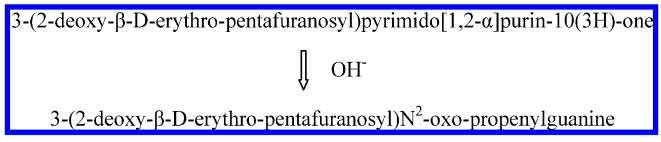
After incubation at 55 °C for at least 60 min, sodium acetate was added to the samples, allowing protein precipitation to the bottom by centrifugation. The supernatant containing DNA was transferred to another tube, and isopropyl alcohol was added to facilitate the precipitation of DNA. After some washes with ethanol (70%), DNA was collected and dissolved in low-TE and finally stored at −20 °C.

Preparation of the Reference M_1dG Adduct Standards. Two reference adduct standards using MDA or H_2O_2 were prepared. Calf thymus (CT) DNA or leukocyte DNA from a blood donor was treated with 10 mM MDA according to published conditions (*33, 34*). Epithelial lung carcinoma cell line A549 was exposed to 100 μM H_2O_2. MDA-treated CT-DNA was then diluted with untreated CT-DNA to obtain decreasing levels of the reference adduct standard to generate a calibration curve.

MALDI-TOF-MS Analysis. DNA adducts also were detected in MDA-treated CT-DNA by mass spectrometry (Voyager DE STR from Applied Biosystems, Framingham, MA) through the following sequence of steps: (1) reaction of DNA with $NaBH_4$ (*35*) followed by precipitation with isopropanol; (2) digestion with snake venom phosphodiesterase and nuclease P1; (3) extraction of DNA adducts that are less polar than normal nucleotides (NNs) on an OASIS cartridge (Waters Corp.); (4) tagging with an isotopologue pair of benzoylhistamines (d_0 and d_4) in a phosphate-specific labeling reaction in the presence of carbodiimide (*36*); (5) removal of residual reagents by ion exchange solid-phase extraction; (6) resolution of tagged adducts by capillary reversed-phase HPLC with

a collection of drops onto a MALDI plate; (7) addition of matrix (α-cyano-4-hydroxycinnamic acid); and (8) analysis by MALDI-TOF-MS.

M_1dG Adduct Analysis. As previously mentioned, the production of MDA has been demonstrated in vivo on rats exposed to FA (*21, 30*). Thus, we measured the levels of M_1dG adducts in FA-exposed workers and controls using our previously described method (*34*). DNA (1−2 μg) was digested by micrococcal nuclease and spleen phosphodiesterase. Hydrolyzed samples were treated with nuclease P1 (2.5 μg) for 30 min at 37 °C. The nuclease P1 treated samples were incubated with 15−25 μCi of [γ-^{32}P]-ATP and T4-polynucleotide kinase (0.75 U/μL) to generate labeled M_1dG adducts. Samples were applied to the origin of chromatograms and developed with 0.35 $MgCl_2$ up to 2.0 cm on a filter paper wick. Plates were developed in the opposite direction with 2.1 M lithium formate and 3.75 M urea, pH 3.75, and then run at the right angle to the previous development with 0.24 M sodium phosphate and 2.4 M urea, pH 6.4. Detection and quantification of M_1dG adducts and total nucleotides were obtained by storage phosphor imaging techniques employing intensifying screens from Molecular Dynamics (Sunnyvale, CA) for 0.20−48 h. The screens were scanned using a Typhoon 9210 (Amersham, Buckinghamshire, United Kingdom). The software used to process the data was ImageQuant (Molecular Dynamics). After background subtraction, the levels of M_1dG adducts were expressed such as relative adduct labeling (RAL) = screen pixel in adducted nucleotides/screen pixel in total NNs. To calculate the levels of total NNs, aliquots of hydrolyzed DNA were appropriately diluted and reacted in the mixtures used for M_1dG adduct labeling. The obtained ^{32}P-labeled total nucleotides were separated on Merck PEI-cellulose TLC plates using 280 mM $(NH_4)_2SO_4$ and 50 mM NaH_2PO_4. The values measured for the M_1dG adducts were corrected across experiments based on the recovery of the internal standard. Cochromatography of leukocyte DNA samples with MDA treated CT standard was performed to identify the M_1dG adducts detected in the chromatograms of human samples using 2.1 M lithium formate and 3.75 M urea, pH 3.75, and 0.24 M sodium phosphate and 2.4 M urea, pH 6.4, or 0.24 M sodium phosphate and 2.7 M urea, pH 6.4. Furthermore, because previous studies have shown the hydrolytic ring-opening of M_1dG at pH 11.7 (*37, 38*), we have also decided to evaluate whether the alkaline hydrolysis can induce the autoradiography disappearance of leukocyte M_1dG adduct spots. Thus, M_1dG adduct spots, which have been previously identified in the chromatograms of volunteers, were excised and incubated with−without increased concentrations of ammonium hydroxide (0.1, 0.5, 1.0, and 2.0 M, pH 11.7) for 10 min to induce the following ring-opening reaction:



After alkaline hydrolysis, the samples were transferred to the same chromatogram and run side by side with 0.24 M sodium phosphate and 2.4 M urea, pH 6.4. Our results indicate that the alkaline hydrolysis of M_1dG induces the autoradiography disappearance of M_1dG adduct spots, supporting the identity of leukocyte adduct spots such as M_1dG.

Statistical Analysis. Statistical analyses were performed on log-transformed M_1dG data to stabilize the variance and normalize the distribution of adducts. FA-exposed workers and controls were a priori grouped according to tertiles for levels of personal FA exposure. Mean concentrations of FA and M_1dG adduct levels across variable levels were compared by analysis of covariance, introducing into each model terms for sex, age (continuous), smoking, exposure status, and air-FA measurements, as appropriate. *Post hoc* Dunnett tests were performed for multiple comparisons among variable levels. A p value of <0.05 (two-tailed) was considered significant for all of the tests. The data were analyzed using SPSS 13.0 (SPSS, United States).

Table 1. Distributions and Percentage of Epidemiological Characteristics and the Average Levels of FA (μg/m^3) According to Study Variables

		FA		
	n^a	mean ± SE	median	p
gender				
male	22	97.3 ± 40.9	25.6	
female	53	67.2 ± 13.0	29.7	0.769
age				
<30 years	24	66.1 ± 24.2	27.0	
30−39 years	23	94.8 ± 34.4	29.0	
>39 years	28	60.0 ± 20.5	27.4	0.010
smoking				
not smokers	57	67.2 ± 13.1	29.7	
smokers	18	136.9 ± 51.1	26.2	0.065
exposure status				
controls[b]	32	27.7 ± 2.5	25.6	
pathologists				
working in reduction room	19	212.4 ± 47.0	128.6	<0.001
working in other areas	24	32.4 ± 6.1	23.3	0.984

[a] Some figures do not add up to the total because of missing values. [b] Reference level.

Table 2. Distributions and Percentage of Epidemiological Characteristics and the Mean Levels of M_1dG Adducts *Per* 10^8 NNs According to Study Variables

		M_1dG adducts		
	n^a	mean ± SE	median	p^c
status				
controls	20	2.4 ± 0.3	1.8	
reduction room pathologists	20	5.7 ± 1.3	3.3	0.045
gender				
male	12	3.8 ± 1.3	2.4	
female	28	4.1 ± 0.9	2.6	0.345
age				
<30 years	14	3.2 ± 1.1	1.5	
30−39 years	13	4.8 ± 1.5	2.8	
>39 years	13	4.2 ± 1.2	3.0	0.990
smoking				
not smokers	27	3.8 ± 0.9	2.1	
smokers	13	4.5 ± 1.3	3.0	0.494
air-FA				
<22 μg/m^3[b]	13	2.3 ± 0.44	1.8	
23−66 μg/m^3	13	2.7 ± 0.55	2.0	0.775
>66 μg/m^3	13	7.3 ± 1.90	4.2	0.018

[a] Some figures do not add up to the total because of missing values. [b] Reference level. [c] Adjusted by sex, age, and smoking.

Results

The 76 subjects were subgrouped into (a) pathologists working in reduction rooms, (b) pathologists working in other laboratory areas of the three Italian pathology wards, and (c) controls. The frequency of epidemiological characteristics, personal air-FA concentrations, and M_1dG adduct levels are reported in Tables 1 and 2, respectively.

FA in Personal Air. Table 1 shows a general description of air-FA concentrations (μg/m^3) measured with the personal samplers. Our results show that the subjects working in the reduction rooms showed personal air-FA values 7.7 times higher than those of controls ($p < 0.001$) and 6.5-fold higher values than those of pathologists working in other laboratory areas ($p < 0.001$).

Among the reduction room workers, four subjects showed values higher than the limit set by ACGIH at 370 μg/m^3. The levels of air-FA measurements tended to be increased in smokers. A positive and statistically significant correlation

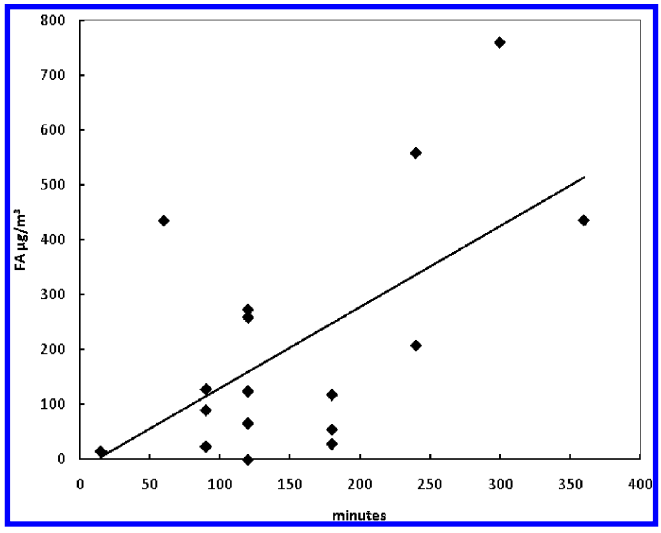


Figure 1. Correlation between air-FA and minutes spent in reduction rooms in subjects "truly exposed".

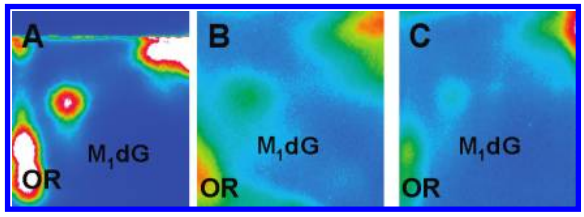


Figure 2. M_1dG adduct pattern in 10 mM MDA-treated human leukocyte DNA (A), in pathologists working in the reduction rooms (B), and in controls (C).

between the minutes spent in the reduction room and the level of air-FA was also found ($r = 0.599$, $p = 0.014$) (Figure 1).

Reference M_1dG Adduct Standards. The capability of MDA treatment to induce in vitro the formation of M_1dG adducts in CT-DNA was evaluated (*34*). A significant increment in the formation of M_1dG adducts was found in MDA-treated DNA relative to control DNA ($p < 0.001$). The average levels of M_1dG adducts per 10^6 nn ± SE were 0.3 ± 0.1, 1.6 ± 0.2, and 5.0 ± 0.4 in 1, 4, and 10 mM MDA-treated DNA, respectively, while a mean level of 0.1 M_1dG adducts per 10^6 NN (±0.01) was detected in untreated DNA. A calibration curve was generated ($R^2 = 0.99$).

We then analyzed whether MDA treatment was capable of inducing M_1dG in human leukocyte DNA (Figure 2A). A significant increase in M_1dG adducts was found relative to control DNA ($p < 0.001$). The average level of M_1dG adducts per 10^6 NN ± SE was 2.2 ± 0.6 and 0.02 ± 0.01 in MDA-treated and untreated DNA, respectively. The presence of a background adduct spot in the untreated samples is in keeping with previous studies reporting endogenous levels of M_1dG adducts in control DNA (*33, 39*). This adduct was previously identified as M_1dG using different techniques (*33, 39, 40*). The presence of MDA adducts in the MDA-treated CT-DNA (10 mM MDA) was also confirmed by us, subjecting it to analysis by mass spectrometry, giving the results shown in Figure 3. As seen, six MDA DNA adducts apparently were identified, including one (M3mdC) that has not been reported before.

Finally, the induction from free radicals of the same DNA lesion in an in vitro system by incubating epithelial lung carcinoma cell line A549 with H_2O_2 was also analyzed. Our

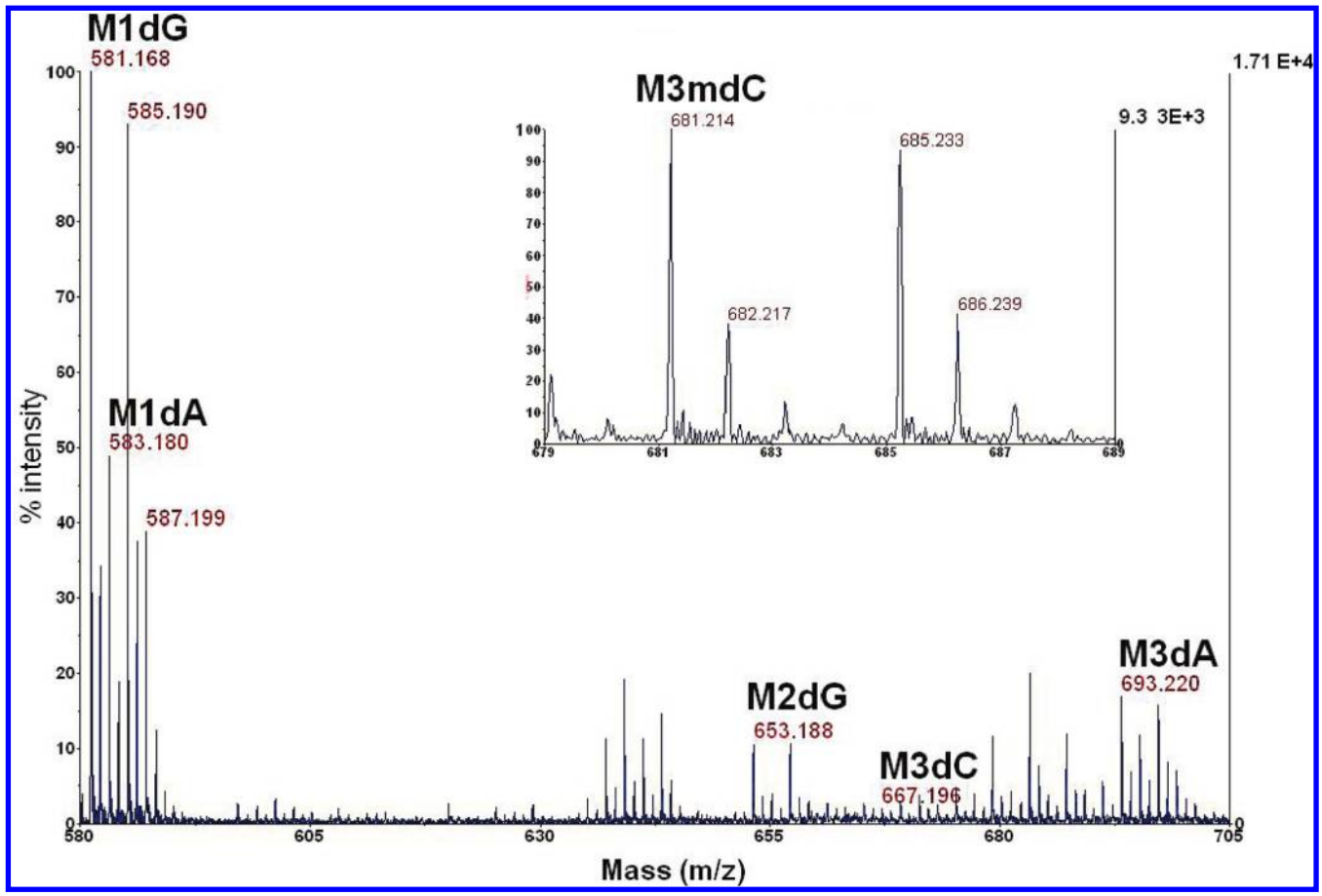


Figure 3. Detection of DNA adducts after mass tagging by MALDI-TOF-MS in MDA-treated, $NaBH_4$-reduced CT-DNA. Relative to the accurate masses that are seen in the spectrum from one spot, the exact masses are as follows using the M nomenclature of Goda and Marnett (*35*): M1dG, 581.166; M1dA, 583.182; M2dG, 653.187; M3dC, 667.192; M3dA, 693.219; and in the inset (from another MALDI spot), M3mdC, 681.207.

Table 3. Distributions and Percentage of Epidemiological Characteristics Mean Levels of M_1dG per 10^8 NNs According to Smoking

	M_1dG adducts nonsmokers				M_1dG adducts smokers			
	n^a	mean ± SE	median	p^c	n^a	mean ± SE	median	p^c
gender								
male	6	3.1 ± 1.0	2.3		6	4.5 ± 2.5	2.4	
female	21	4.0 ± 1.1	2.1	0.785	7	4.5 ± 1.3	3.9	0.696
age								
<30 years	8	2.0 ± 0.5	1.65		6	4.8 ± 2.5	3.2	
30−39 years	11	4.9 ± 1.8	2.8		2	4.5 ± 1.6	4.5	
>39 years	8	4.2 ± 1.7	2.6	0.304	5	4.1 ± 1.8	2.0	0.701
air-FA								
<22 $\mu g/m^3$ [b]	9	2.1 ± 0.4	1.8		4	2.7 ± 1.2	1.9	
23−66 $\mu g/m^3$	10	2.6 ± 0.7	1.7	0.745	3	3.0 ± 0.5	2.0	0.883
>66 $\mu g/m^3$	7	8.0 ± 2.9	4.2	0.009	6	7.3 ± 1.9	4.2	0.647

[a] Some figures do not add up to the total because of missing values. [b] Reference level. [c] Adjusted by sex and age.

findings showed that treatment with 100 μM H_2O_2 induced a significant increase in M_1dG adducts in the lung carcinoma cells relative to the unexposed cells ($p < 0.001$). The mean level of M_1dG adducts per 10^6 NN was 0.25 ± 0.09 and 0.07 ± 0.01 in H_2O_2-treated and untreated cells, respectively.

M_1dG Adducts in the Leukocytes of Pathologists. M_1dG adduct analysis was focused on the pathologists that were exposed to FA and on controls. Figure 2 reports the pattern of M_1dG adducts detected in the chromatograms of the study volunteers. The intensity of M_1dG adducts was generally stronger in the chromatograms of FA-exposed pathologists as compared with those of controls (Figure 2B vs C).

The cochromatography confirmed the presence of M_1dG in leukocyte DNA. Then, alkaline hydrolysis experiments showed that the autoradiography disappearance of leukocyte M_1dG adducts was associated with increased OH^- concentrations, supporting the identity of leukocyte adduct spots such as M_1dG adducts.

Any differences were found taking into account gender and age in the 40 subjects considered for M_1dG analysis (Table 2). Table 2 also shows mean levels of M_1dG adducts in the subgroups B and C of Figure 2. The difference for M_1dG adduct levels between the pathologists working in the reduction rooms (5.7 M_1dG adducts per 10^8 nn ±1.3) and the controls (2.4 M_1dG adducts per 10^8 ± 0.3) was statistically significant ($p = 0.021$) and persisted after adjusting for sex, age, and smoking ($p = 0.045$). The association with M_1dG adducts became stronger when the evaluation of air-FA exposure was based on personal samplers ($p = 0.003$) and persisted after adjusting for sex, age, and smoking ($p = 0.018$).

When the study population was subgrouped according to smoking, a general direct relationship between M_1dG adduct levels and personal air-FA exposure was observed. Nevertheless, a statistically significant higher level of M_1dG adducts ($p = 0.009$) was recorded when exposed to higher concentrations of air-FA only in nonsmokers (Table 3), probably due to the small number of analyzed subjects. These findings were confirmed by subgrouping smokers and nonsmokers in only two levels of air-FA exposure: a larger group (10 + 9 nonsmokers and 4 + 3 smokers) less exposed to FA (≤66 $\mu g/m^3$) and the same groups (7 nonsmokers and 6 smokers) more heavily exposed to air-FA (>66 $\mu g/m^3$).

A final statistical analysis was performed by evaluating the correlation between M_1dG adduct and air-FA exposure levels. The Pearson correlation showed a positive role but not significant for air-FA in M_1dG levels in nonsmokers ($r = 0.355$, $p = 0.075$, Figure 4) but not in smokers.

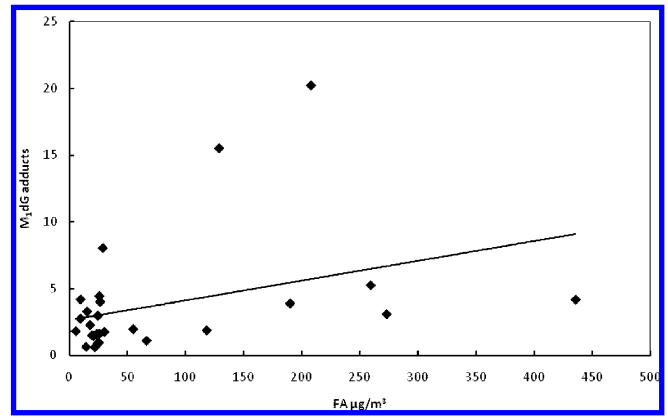


Figure 4. Pearson's correlation between M_1dG adduct level and personal air-FA ($r = 0.355$, $p = 0.075$) in all 27 nonsmokers among the 40 subjects selected for M_1dG.

Discussion

Oxidative stress has been related to the etiopathogenesis of several chronic diseases (aging, hepatic and renal diseases, and cancer) mainly caused by the presence of aldehydes formed by lipids oxidation. LPO is one of the processes induced by oxidative stress (*41, 42*). MDA is the principal and most studied aldehyde produced by polyunsaturated fatty acid peroxidation. In the physiological state, at neutral pH, MDA is present as an enolate anion and shows low chemical reactivity. Nevertheless, this molecule is able to interact with nucleic acid bases to form several different adducts (*43*). Breathed FA can induce oxidative stress through different ways, an example of which is the production increase of enzymes utilized in FA detoxification after FA exposure (*21, 30*). For this reason, we decided to study the relationship between occupational exposure to air-FA and the levels of M_1dG adducts.

The results of the present survey show that about 50% of the pathologists recruited in the three Italian pathology wards were truly, professionally exposed to air-FA. The "real cases" were those working in the "reduction rooms", where FA was directly used to fix the biological tissues (see Figure 1). Statistical analysis in Table 1 confirms the "real cases" as the sole subgroup with higher personal air-FA levels if compared to all of the other subjects enrolled in both the pathologists and the control subgroups. This evidence emphasizes how the laboratories where tissue reduction is performed are the most risky in pathology departments where the best preventive measures are desirable.

Four subjects working in the "reduction rooms" have shown personal air-FA levels superior to the ACGIH limit (0.370 mg/m^3). Given the good environmental working conditions of these workers and the relatively low FA levels shown by the other workers operating in the "reduction room", we can assume that these high values could depend on improper work behavior rather than on poor environmental working conditions. However, some authors described workers exposed to similar or higher FA pollution levels if compared to the ACGIH limit (*15, 16, 44*). Thus, air-FA does not represents an indistinct risk factor for all of the workers in the pathology wards (doctors, students, technicians, etc.) but "only" a preventive item connected to a specific workstation.

Then, we focused the measurements of M_1dG adducts on the group of pathologists really exposed to FA and on controls. Our results show that working in a reduction room was

associated with increased leukocyte M_1dG adduct levels; indeed, the levels of M_1dG adducts from air-FA of professionally exposed pathologists were significantly higher than those of controls (Table 2). This association also persisted when personal air samplers were used to measure the extent of air-FA exposure. Interestingly, the analysis of the dose−response relationship shows an increased adduct formation only in workers exposed to external air-FA levels higher than 66 μg/m^3 but not in subjects exposed to lower air-FA values. In addition, the effect of air-FA exposure was more evident in the group of nonsmokers, suggesting that the role of FA exposure in the M_1dG adducts may have been masked by smoking (Table 3).

FA is the main product used in the pathology laboratories where tissue reduction is performed; thus, it can be involved in the induction of increased levels of M_1dG adducts of the group of laboratory workers exposed to FA. FA can induce increased levels of oxidative stress and enhanced formation of ROS by different ways, including the activation of oxidases and the inhibition of scavenger systems. For instance, FA is a substrate for the action of cytochrome P-450 monooxygenase system II E1 isozyme and can be oxidized by peroxidase, aldehyde oxidase, and xanthine oxidase with subsequent ROS formation (*22*). A pathway independent from MDA production can also be involved in the formation of M_1dG adducts. Indeed, Dedon (*45*) proposed a mechanism in which M_1dG can also be formed directly upon ROS exposure through the formation of base propenal. In addition, impaired antioxidant activities of enzymes, in metabolic detoxification of oxidative byproduct, have also been reported in experimental animals treated with FA (*22, 46*). Finally, FA exposure can lead to inflammation and to a consequent excess of oxidative stress (*47*). Activated macrophages and neutrophils can release ROS, such as hydrogen peroxide and hypochlorite acid, which can induce LPO and MDA formation. Indeed, we have recently shown that ROS generated by activated inflammatory neutrophils are involved in M_1dG adduct formation (*48*).

Our findings can be useful to characterize the risk, at least in terms of DNA damage, experienced from the subjects working in the "reduction rooms" of the pathology wards. However, predictive values of biomarkers, especially those from internal doses and early biological effects, are quite limited to assess individual risk. This is because the processes that lead from exposure to disease are affected by many factors, many of which are unknown or whose real impact is not estimable (e.g., individual genetic profile, age, diet, lifestyle and health status, mode of exposure, etc.). Conversely, the predictive values of biomarkers of DNA damage in a given population remain of relevant importance at the level of public health concern (*49, 50*).

Finally, the role of tobacco smoke habits was investigated. Cigarette smoke contains a broad range of carcinogens and ROS derived from tobacco pyrolysis products that can lead to M_1dG adduct formation. The levels of M_1dG adducts tended to increase in smokers with respect to nonsmokers but without reaching a statistical significance (Table 2). Moreover, the association of M_1dG adducts with smoking tended to be more present in subjects exposed to air-FA exposure levels lower than 66 μg/m^3 than in individuals exposed to higher air-FA concentrations (Table 3).

The relationship between smoking and M_1dG adducts was previously examined by us in two hospital-based studies and a higher amount of DNA lesions was found in the bronchi and larynx of smokers (*27, 28*). The levels of endogenous DNA damage were also increased in lung cancer cases with respect to controls, but smokers and lung cancer cases with levels of MDA−DNA adducts above the population median also had reduced survival (*28*). Similar relationships were observed in the leukocytes and oral mucosa of smokers (*29, 51*), but other authors reported no differences for tobacco smoking in breast and colon mucosa (*26, 52*). Previous experimental studies have also indicated that cigarette smoking causes the elevations of urinary F_2-isoprostanes, an indicator of oxidative stress (*53, 54*).

In summary, our study shows that working in the reduction rooms of pathology wards and exposure to air-FA concentrations higher than 66 μg/m^3 are associated with increased levels of MDA−dG adducts. Taking into account that the TLV-TWA level for FA is almost six times higher than 66 μg/m^3 (370 μg/m^3), we must consider this last biological finding important knowledge for future preventive actions aimed at these kinds of workers. The present work lays the basis for further investigations with increased statistical power and based on less invasive and more reliable surrogate target sampling, for example, nasal epithelial cells instead of venous blood, to assess the biological effects on tissues more affected by exposure to FA.

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