Short Communication

In Vitro *Synthesis of 1,N^6-Etheno-2′-deoxyadenosine and 1,N^2-Etheno-2′-deoxyguanosine by 2,4-dinitrophenol and 1,3-dinitropyrene in Presence of a Bacterial Nitroreductase*

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ABSTRACT: The formation of covalent nitro-PAH DNA adducts and nitro-PAH mediated oxidative lesions are two possible mechanisms for the initiation of nitro-PAH carcinogenesis. Sixty-minute incubation of 1,3-dinitropyrene (100 μM) or 1,4-dinitrophenol (100 μM) with a mixture of 150 μM NADH, 0.5 units of *E. coli* nitroreductase, 100 μM linoleic acid, 0.5 mM ferrous iron, and 100 μM 2′-deoxyadenosine (2′-dA) or 100 μM 2′-deoxyguanosine (2′-dG) were analyzed by liquid chromatography multistage mass spectrometry. Mixtures of 1,N^6-etheno-2′-deoxyadenosine (εdA) plus 4-oxo-2-nonenal (4-ONE) and 1,N^2-etheno-2′-deoxyguanosine (εdG) plus 4-ONE could be detected from 2′-dA and 2′-dG, respectively. Addition of 2% propanol inhibited the formation of etheno adducts. Analyses of disappearance kinetics of dA and dG showed that dG was more rapidly eliminated than does dA (t[1/2] = 23.3 min and 98.3 min for dG and dA, respectively). Curves of formation kinetics revealed that the peak of εdG was at 55.6 min while that of εdA was at 186.9 min. These peaks represented 1.43% and 1.25% of the original dG and dA, respectively. In both cases, the peaks were followed by rapid degradations of etheno adducts. The results, obtained in this system, do not allow any extrapolation to realistic cellular responses; nevertheless, these data questioned the validity of the use of unsubstituted etheno adducts as reliable oxidative stress and nitro-PAH exposure biomarkers. © 2007 Wiley Periodicals, Inc. Environ Toxicol 22: 222–227, 2007.

Keywords: etheno-DNA adducts; nitro aromatic compounds; environmental exposure; carcinogenesis

INTRODUCTION

The occurrence of nitro aromatic compounds (NACs), mainly nitro polycyclic aromatic hydrocarbons (nitro-PAHs) and nitrophenols, in the ambient air is a matter of concern for the possible impact of these compounds on human health (Atkinson and Arey, 1994). This is linked to their high direct mutagenicity. The presence of nitro-PAHs is due both to direct traffic emission and to atmospheric formation (Vione et al., 2005a). The compound 2-nitrofluoranthene (2-NF) is by far the most abundant nitro-PAH in atmospheric particles, while the nitronaphthalenes and alkylnitronaphathalenes make up 80% of the total nitro-PAHs measured in the gas phase at a concentration ranging from 50 to 150 pg m^{-3} (Reisen et al., 2005). The International Agency for Research on Cancer has assessed that 1,8-dinitropyrene and 1,6-dinitropyrene were possibly carcinogenic to humans (group 2B). Fortunately, 1,3-, 1,6-, 1,8-dinitropyrene are encountered at lower levels than 2-NF, nitro- and

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alkylnitronaphthalenes (100 folds, Tang et al., 2005). Nitrophenols, such as 2,4-dinitrophenol, are also significant atmospheric pollutants. The molecule 2,4-dinitrophenol has been detected at μg L^{-1} levels in rain (Asman et al., 2005) and is thought to be a contributing factor of the forest decline. Surprisingly, its genotoxic capacity has not been fully addressed yet. The occurrence of this compound is thought to result from the nitration of mononitrophenols in the aqueous phase of the atmosphere (Vione et al., 2005b).

Nitroreduction is regarded as a relevant pathway in the metabolic activation of nitro-PAHs to mutagenic species (Carroll et al., 2002). Nitroreduction of nitro-PAHs, catalyzed by two-electron transfer reaction (e.g., diaphorase), yields *N*-hydroxy arylamines. These molecules react, directly or upon esterification of the *N*-hydroxy function, with DNA to form covalent adducts (Ritter et al., 2002). The reduction rate increases with the number and the position of the nitro groups (Fu et al., 1998). In contrast to two-electron reaction, single electron reactions of flavoenzyme electron transferase (e.g. NADPH: cytochrome P-450 reductase or ferredoxin: $NADP^+$ reductase) can initiate a redox cycling of nitrocompounds and challenge the formation of *N*-hydroxylamine. This type of reactions promotes also the formation of reactive oxygen species (ROS) and the ROS induce oxidative DNA damage (Murata et al., 2004).

Unsubstituted etheno-DNA adducts (ε-adducts), such as 1,N^6-etheno-2′-deoxyadenosine (εdA), 1,N^2-etheno-2′-deoxyguanosine (εdG), and 3,N^4-etheno-2′deoxycytosine (εdC) are highly mutagenic, because they are chemically stable and are slowly removed in case of impairment or imbalance of cellular DNA repair pathways. The persistence of such DNA adducts might lead to the increase in mutations and genomic instability (Bartsh and Nair, 2005; Bolt, 2005). These chemicals were originally considered to originate from exposure to environmental carcinogens, such as the occupational carcinogen vinyl chloride. They are now recognized to be produced endogenously by lipid peroxidation. Lately, ε-adducts have been examined as useful markers to assess oxidative stress and lipid peroxidation during the early stages of carcinogenesis, particularly during the inflammatory process. However, the direct link between the exposure to environmental NACs and the formation of ε-adducts through a lipid oxidation process has not been clearly evidenced until now. Thus, the major aims of this work were the following:

i. To assess the ubiquity of the *in vitro* 1,N^6-etheno-2′-deoxyadenoside and 1,N^2-ethano-2′-deoxyguanosine formation upon the presence of nitroreductase and NACs.
ii. To study the kinetics of elimination of 2′-deoxyadenosine (2′-dA) and 2′-deoxyguanosine (2′-dG) and the kinetics of production of εdA and εdG.
iii. To discuss the possibility to use ε-adducts as NACs exposure biomarkers.

MATERIALS AND METHODS

Chemicals

2,4-Dinitrophenol (purity >99%), β-nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH, purity >98%), nitroreductase (*E. coli*, 2.46 units/mg protein), linoleic acid (purity >99%), 1,N^6-etheno-2′-deoxyadenosine, 4-hydroxy-2-nonenal (4-HNE) dimethylacetal were purchased from Sigma-Aldrich (St. Quentin Fallavier, France). 2′-Deoxyguanosine (purity >99%), 2′-deoxyadenosine (purity >99%), sodium phosphate dibasic (purity >99%), and potassium phosphate monobasic (purity >99%) from Acros Organics (Noisy le Grand, France). All reactants were used without further purification. Methanol was LiChrosolv gradient grade from Merck (Fontenay Sous Bois, France). Solutions and HPLC eluents were prepared with Milli-Q water. $FeSO_4$ (puriss.p.a.) and formic acid (>98%) were from Fluka (St. Quentin Fallavier).

Synthesis of 4-Oxo-2-nonenal

4-Oxo-2-nonenal (4-ONE) dimethylacetal was prepared by MnO_2 oxidation of 4-hydroxy-2-nonenal (4-HNE) dimethylacetal. The latter was synthesized according to the method of Esterbauer and Weger (1967). 4-ONE was obtained by acid treatment of 4-ONE dimethylacetal.

Synthesis of 1,N^2-etheno-2′-deoxyguanosine (εdG)

The compound εdG was synthesized according to the procedure of Sattsangi et al. (1977) by using a phosphate buffer (0.1 M; pH 6.4). The molecule εdG was isolated by collecting fractions during HPLC for further characterization by nuclear magnetic resonance (NMR).

Analytical Determinations

The identifications of dA, dG, εdA, εdG, and 4-ONE were carried out by liquid chromatography multistage mass spectrometry (LC/MS^2). Positive electrospray ((+) ESP) was performed on an Esquire 6000 ion trap system (Bruker, Bremen, Germany), with a potential of 4 kV applied to the electrospray needle. Nitrogen was used as drying and nebulizing gas. The capillary temperature was held at 250 °C. Full scanning analysis was performed in the range of 100–500 *m/z*. The relative collision energy was set at 30% of the maximum (1 V). The $[M+H]^+$ ion at *m/z* 276, *m/z* 292, *m/z* 252, and *m/z* 268 was selected as the precursor ion for εdA, εdG, dA, and dG, respectively. All compounds showed the same fragmentation pattern. Daughter ion spectra were only detected as one intense base peak at *m/z* 160, *m/z* 176, *m/z* 136, and *m/z* 152 for εdA, εdG, dA and dG, respectively. All fragments corresponded to the loss of the neutral 2′-deoxyribose moiety (116 uma) formed by the rupture of the N-glycoside bond. The MS^2 mass spectrum of 4-oxo-2-

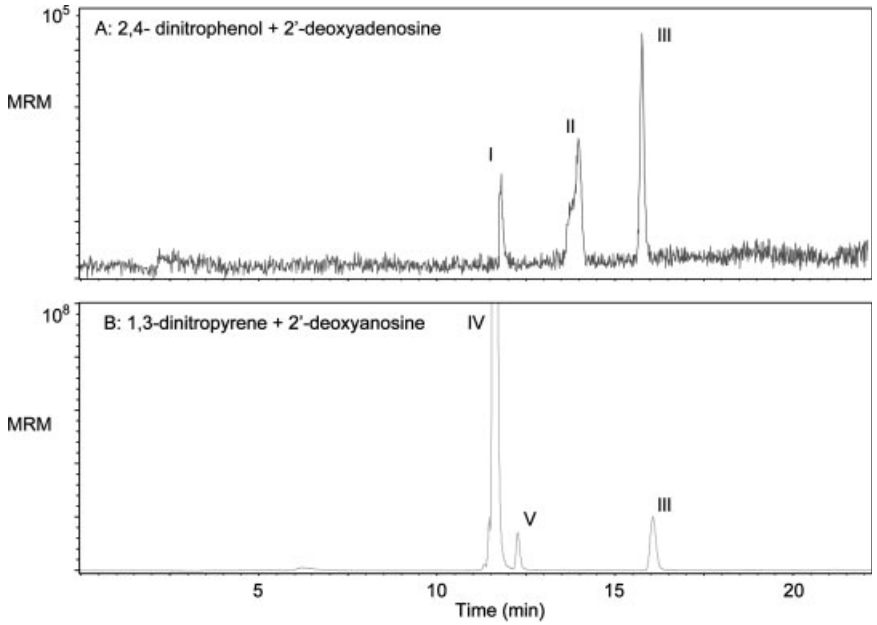

Fig. 1. LC/MS^2 chromatograms after treatment of two NACs with a bacterial nitroreductase. A. 2,4-Dinitrophenol and 2′-dA; B. 1,3-dinitropyrene and 2′-dG. NACs and dA or dG were incubated at 37 °C for 60 min with a mix that included linoleic acid, *E. coli* nitroreductase, NADH, and Fe^{2+} ions. Identified peaks: (I) 2′-deoxyadenosine; (II) 1,N^6-etheno-2′-deoxyadenosine; (III) 4-oxo-2-noneal; (IV) 2′-deoxyguanosine; (V) 1,N^2-etheno-2′-deoxyguanosine.

nonenal did not depict intense fragmentation ion, and this chemical was only characterized by the $[M+H]^+$ ion at *m/z* 155. Multiple reaction monitoring was used for quantification purposes. The *m/z* 292 → 176, *m/z* 276 → 160, *m/z* 252 → 136, and *m/z* 268 → 152 transitions were monitored for εdG, εdA, dA, and dG, respectively. The detection limits ($S/N = 3$) for εdA and εdG were 1 and 1.5 ng mL^{-1} corresponding to 10 and 15 pg injected on column. The coefficient of variation during the injection of 20 standard samples was 5.5%. Quantification by means of an external calibration procedure was allowed, since ion suppression was not expected with synthetically made samples.

Gradient elutions were achieved in the linear mode and chromatography was performed with an Elite LabChrom system (VWR, France). The analytical separation was carried out with a Metachem C-18 column (150 × 2 mm^2 i.d., 3 μm) at a flow rate of 0.2 mL min^{-1}. The mobile phase consisted of a binary mixture of solvents A (methanol) and B (0.1% formic acid in H_2O). The gradient was performed from 100% B to 50% A in 15 min and from 50% A to 0% A in 5 min. The return to the initial conditions was accomplished in 5 min.

Enzymatic Assays

Biotransformation assays with the nitroreductase were performed in 100 mL Erlenmeyer flasks wrapped with an aluminum foil and in aqueous solution saturated with oxygen at pH = 7 and at 37 °C. Each Erlenmeyer flask contained 2,4-dinitrophenol (100 μM) or 1,3-dinitropyrene (100 μM), NADH (150 μM), nitroreductase (0.5 native units), 2′-dA or 2′-dG (100 μM), linoleic acid (100 μM), and ferrous iron (0.5 mM) in a final volume of 50 mL potassium phosphate buffer (50 mM, pH = 7). A control without the enzyme was prepared for each experiment. An additional control involving the Fenton's reaction (Fe^{2+}/H_2O_2) as a hydroxyl radical source and in the absence of the enzyme was also prepared for each experiment. Aliquots (1 mL) from the liquid phase were withdrawn periodically for analysis by LC/MS^2.

RESULTS

When the experiments were carried out in the absence of the nitroreductase enzyme (results not shown), dA and dG remained stable. It could be inferred that reactions, proceeding through electron-transfer processes by means of Fe^{2+} ions, were of a very minor significance in our experimental system. Incubation of 1,3-dinitropyrene or 2,4-dinitrophenol in neutral phosphate buffer (pH 7.0) for 1 h at 37 °C in presence of dA, nitroreductase, NADH, Fe^{II}, and linoleic acid led to a complex mixture of transformation products. Among them, residual dA (I), εdG (II), and 4-ONE (III) were fully identified by comparison of their chromatographic and mass spectrometry profiles with that of authentic standards as depicted in Figure 1(A). Using a gradient

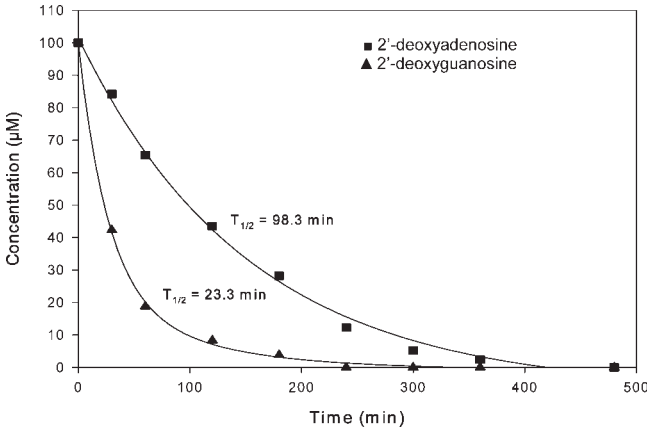

Fig. 2. Degradation kinetic profiles of 2′-deoxyguanosine (dG) and 2′-deoxyadenosine (dA). Degradation profiles followed first-order kinetics. Calculated half lives were 23.3 min and 98.3 min for dG and dA, respectively.

elution profile, LC/MS analysis of the products from the reaction between 1,3-dinitropyrene and 2,4-dinitrophenol with dG in presence of nitroreductase, NADH, Fe^{II}, and linoleic acid revealed after 1-h incubation time the presence of εdG (V) in the reaction mixture together with residual amounts of dG (IV) and 4-ONE (III), as illustrated in Figure 1(B). The addition of 2-propanol (2% v/v), a well-known hydroxyl radical scavenger, resulted in the complete inhibition of the formation of ε-adducts. Conversely, when another source of hydroxyl radicals, such as the Fenton's reagent (Fe^{2+}/H_2O_2), was used in the absence of the nitroreductase enzyme, εdA and εdG were formed to a greater extent (results not shown). These results underline the major role of hydroxyl radicals in the degradation of nucleosides. The occurrence of 4-ONE, described as the principal breakdown product of linoleic acid hydroperoxide (Ridgen et al., 1999), provides evidences for the effective oxidation of linoleic acid during the reaction time.

The kinetics of disappearance of dA and dG and formation of εdA and εdG were followed by LC/MS^2 as illustrated in Figures 2 and 3. 2,4-Dinitrophenol was used as the NAC probe, since higher amounts of ε-adducts were obtained as compared to nitro-PAHs because of higher hydrosolubility of this chemical. A 240-min incubation time was required for the total removal of dG, meanwhile the total disappearance of dA occurred after 480 min. Thus, the calculated half lives were 23.3 min and 98.3 min for dG and dA, respectively. Guanine has been shown to be the most susceptible DNA target to a wide variety of oxidation reactions mediated by hydroxyl radicals and singlet oxygen. This property might explain the faster degradation rate of dG as compared to dA (Cadet et al., 1999). The formation kinetics of εdA and εdG were also assessed. Maximal concentrations were observed after 55.6 min and 186.9 min of incubation time for εdG and εdA, respectively. These concentration peaks only accounted for 1.43% and 1.25% of the initial concentrations of dG and dA, respectively. Thus, the ε-adduct formation was clearly a minor pathway. In addition, the concentrations of both ε-adducts steadily decreased, accounting for further chemical transformations of these compounds.

DISCUSSION

The formation of covalent nitro-PAH-DNA adducts and nitro-PAH mediated oxidative DNA damage are possible mechanisms for the initiation of nitro-PAH carcinogenesis. Results reported in this work provide an additional route that leads to oxidative DNA lesions through a ω-6 polyunsaturated fatty acid peroxidation pathway and the formation of ε-adducts. A possible mechanism of εdA and εdG productions can be proposed as depicted in Figure 4. Nitroreductase catalyzes one electron transfer to NACs and produces an anion radical ($NO_2^{\cdot -}$). Molecular oxygen quenches an electron from this anion radical and converts it back to the parent molecule. During this process, a superoxide radical anion ($O_2^{\cdot -}$) is produced. Then, this radical is dismutated into H_2O_2, which reacts with ferrous ion to yield hydroxyl radicals as the major ROS according to the Fenton reaction and promoting linoleic acid peroxidation. The transition metal Fe^{II} induced the homolytic decomposition of the ω-6 polyunsaturated fatty acid lipid hydroperoxide 13-hydroperoxy-*S*-(*Z*,*E*)-9,11-octadecadienoic acid (13-HPODE) to α,β-unsaturated aldehyde genotoxins such as 4-ONE. The transient species 4-hydroperoxy-2-nonenal was not detected in our experiments, but was recently recognized to be responsible for the unsubstituted etheno-adduct formation (Hwa Lee et al., 2005). Apart from εdA, εdG, and 4-ONE, other intermediates or end-products might be formed, such as

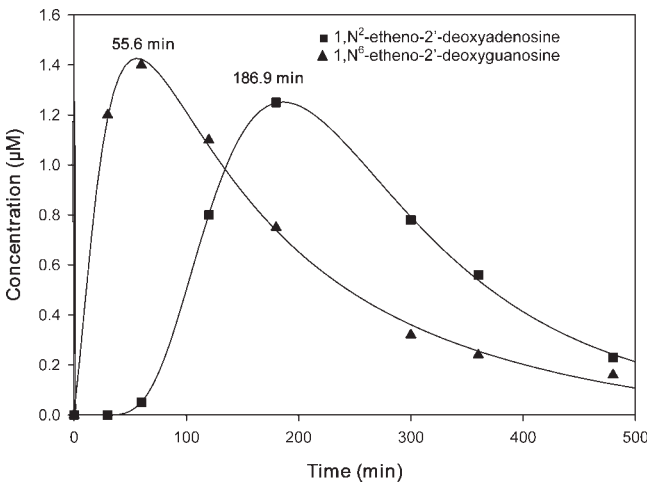

Fig. 3. Formation kinetic profiles of 2-N^6-etheno-2′-deoxyadenosine (εdG) and 2-N^2-etheno-2′-deoxyguanosine (εdA). Formation profiles showed peaks at 55.6 min and 186.9 min for εdG and εdA, respectively. Production peaks were rapidly followed by degradation.

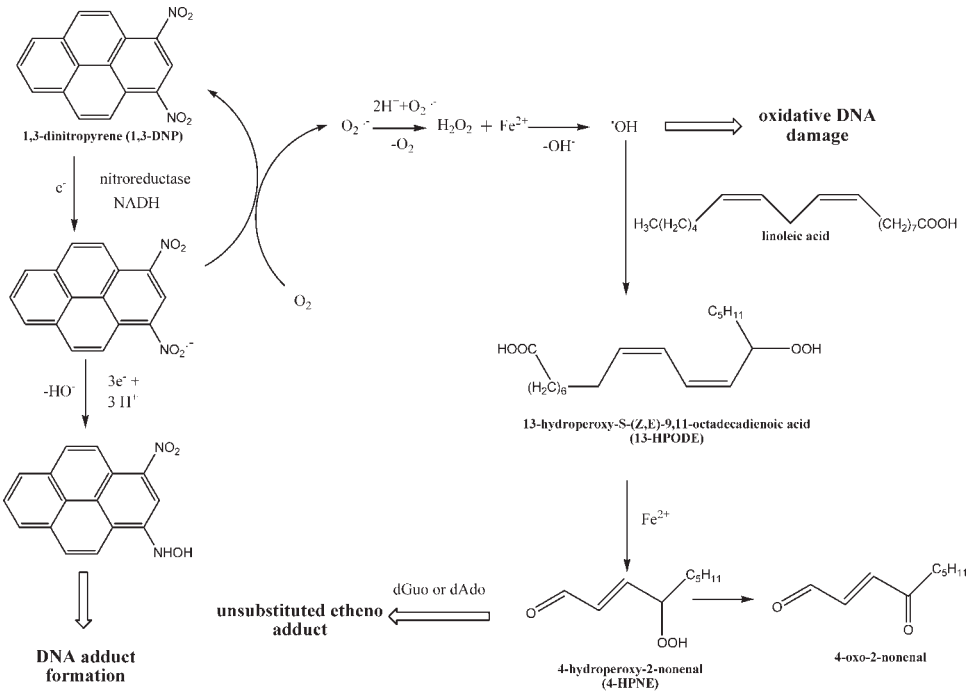

Fig. 4. Proposed mechanism for unsubstituted etheno-adducts formation.

substitituted etheno-adducts or substituted propano-adducts. However, their identification was not investigated, because the main purpose of this work was limited to demonstrate a possible additional oxidative route of DNA damage through lipid peroxidation that could occur in the presence of NACs and bacterial nitroreductase. It can be assumed that the ε-adduct formation pathway could be a common pathway, since redox cycling of NACs is ubiquitous in human cells in the presence of nitroreductases, and since the formation of ε-adducts is possible whatever the structural features of the studied NACs (1,3-dinitropyrene and 2,4-dinitrophenol). Although this pathway is quantitatively a minor pathway, it might contribute to a large extent to the mutagenicity of NACs, because ε-adducts are poorly repaired in human cells, mainly in case of impairment or imbalance of cellular repair pathways (Barbin, 2000).

Recently, ε-adducts have been proposed as potential new tools to study oxidative stress and cancer etiology (Bartsh and Nair, 2000, 2005) due to the difficulties to use 8-oxo-7,8-dihydroguanine (8-oxoG) as a reliable oxidative stress biomarker. 8-oxoG can be easily oxidized during sample preparation (Collins et al., 2004). The potential use of ε-adducts as oxidative stress biomarkers has prompted the development of highly sensitive analytical methods that are based on liquid chromatography coupled to mass spectrometry techniques. These new chromatographic techniques are able to monitor the ε-adducts at trace levels in different body fluids and in DNA (Gonzalez-Reche et al., 2002; Loureiro et al., 2002). It should be underlined that these results are limited to a simulated *in vitro* model and do not allow any extrapolation to realistic cellular responses, particularly because of the poor reactivity of compacted nuclear DNA as compared to free nucleosides in solution. The degradation of ε-adducts observed in this work upon ROS generation and in aqueous solutions, nevertheless, may question the choice of ε-adducts as oxidative stress and NACs exposure biomarkers. This instability deserves further investigation especially in human cells. As 8-oxoG which is readily subjected to oxidation reactions involve singlet oxygen (Duarte et al., 2000), ε-adducts might structurally evolve possibly through oxidation reactions mediated by hydroxyl radicals.

The identification of a relevant oxidative stress biomarker is still under debate. A recent study has focused on the patterns of 4-HNE-induced DNA lesions and has revealed that 1,N^2-propano-2′-deoxyguanosine adduct was found to be the major DNA lesion in cultured human monocytes. The authors stated that this propano-DNA adduct appeared to be poorly repaired in contrast to other etheno-DNA adducts and might represent the best biomarker of genotoxic effects of 4-HNE (Douki et al., 2004).

Three main issues need to be developed in the field of propano- and etheno-adducts: (i) the significance of the different pathways leading to propano and/or etheno-DNA adducts; (ii) the stability of these adducts especially in human cells, and (iii) the influence of impaired or imbalanced DNA repair pathways (base excision repair, nucleotide excision repair, mismatch repair, or AP endonuclease mediated repair) on the steady state levels of etheno- and propano-adducts. Additional studies are required to determine

the importance of the environmental exposure in the production of ROS and DNA reactive aldehydes from lipid peroxidation. Through exocyclic adduct production, DNA can be damaged and can lead to malignancies. Similarly, research for new biomarkers is still required for an early detection and prevention of environmental and occupational carcinogenesis.

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REFERENCES

Asman W, Jorgensen A, Bossi R, Vejrup K, Mogensen B, Glasius R. 2005. Wet deposition of pesticides and nitrophenols at two sites in Denmark: Measurement and contributions from regional sources. Chemosphere 59:1023–1031.

Atkinson R, Arey J. 1994. Atmospheric chemistry of gas-phase polycyclic aromatic hydrocarbons: Formation of tamospheric mutagens. Environ Health Perspect 102(Suppl 4):117–126.

Barbin A. 2000. Etheno-adduct forming chemicals from mutagenicity testing to tumor mutation spectra. Mutat Res 462:55–69.

Bartsh H, Nair J. 2000. New DNA based biomarkers for oxidative stress and cancer chemoprevention studies. Eur J Cancer 36: 1229–1234.

Bartsh H, Nair J. 2005. Accumulation of lipid peroxidation derived DNA lesions: Potential lead markers for chemoprevention of inflammation driven malignancies. Mutat Res 591:34–44.

Bolt H. 2005. Vinyl chloride: A classical industrial toxicant of new interest. Crit Rev Toxicol 35:307–323.

Cadet J, Delatour T, Douki T, Gasparutto D, Pouget JP, Ravanat JL, Sauvaigo S. 1999. Hydroxyl radicals and DNA base damage. Mutat Res 424:9–21.

Carroll D, Warnakulasuriyarachchi D, Nokhbeh M, Lambert I. 2002. Salmonella typhimurium mutagenicity tester strains that over express oxygen insensitive nitroreductase nfsA and nfsB. Mutat Res 501:79–98.

Collins A, Cadet J, Moller L, Poulsen H, Vina J. 2004. Are we sure we know how to measure 8-oxo-7,8-dihydroguaninein DNA from human cells? Arch Biochem Biophys 423:57–65.

Douki T, Odin F, Caillat S, Favier A, Cadet J. 2004. Predominance of the 1, N^2-propano-2′-deoxyguanosine adduct among 4-hydroxy-2-nonenal induced DNA lesions. Free Radic Biol Med 37:62–70.

Duarte V, Gasparutto D, Yamaguchi L, Ravanat J, Martinez G, Medeiros M, Di Mascio P, Cadet J. 2000. Oxaluric acid as the major product of singlet oxygen mediated oxidation of 8-oxo-7, 8-dihydroguanine in DNA. J. Am Chem Soc 122:12622–12628.

Esterbauer H, Weger W. 1967. Uber die wirkungen von aldehyden auf gesunde und maligne zellen 3. Mitt: Synthese von homologen 4-hydroxy-2-nonenal alkenalen II. Monaatsch Chem 98:1994–2000.

Fu P, Von Tungeln L, Chiu L, Zhan D, Deck J, Bucci T, Wang J. 1998. Structure, tumorigenicity, microsomal metabolism and DNA binding of 7-nitrodibenz[*a*,*h*]anthracene. Chem Res Toxicol 11:937–945.

Gonzalez-Reche L, Koch H., Weiß T, Müller J, Drexler H, Angerer J. 2002. Analysis of ethanoguanine adducts in human urine using high performance liquid-chromatography tandem mass spectrometry. Toxicol Lett 134:71–77.

Hwa Lee S, Arora J, Oe T, Blair IA. 2005. 4-Hydroperoxy-2-nonenal induced formation of 1,N^2-etheno-2′-deoxyguanosineadducts. Chem Res Toxicol 18:780–786.

Loureiro A, Marques S, Garcia C, Di Mascio P, Medeiros M. 2002. Development of an on-line liquid chromatography electrospray tandem mass spectrometry assay to quantitatively determine 1,N^2-etheno-2′-deoxyguanosinein DNA. Chem Res Toxicol 15:1302–1308.

Murata M, Ohnishi S, Seike K, Fukuhara K, Miyata N, Kawanishi S. 2004. Oxidative DNA damage induced by carcinogenic dinitropyrenes in the presence of P-450 reductase. Chem Res Toxicol 17:1750–1756.

Reisen F, Arey J. 2005. Atmospheric reactions influence seasonal PAH and nitro-PAH concentrations in the Los Angeles basin. Environ Sci Technol 39:64–73.

Ridgen D, Nakajima M, Wekrli S, Xu K, Blair I. 1999. Covalent modifications to 2′-deoxyguanosine by 4-oxo-2-nonenal, a novel product of lipid peroxidation. Chem Res Toxicol 12:1195–1204.

Ritter C, Culp J, Freeman J, Marques M, Beland F, Malejka-Giganti D. 2002. DNA adducts from nitroreduction of 2,7-dinitrofluoren, a mammary gland carcinogen, catalyzed by rat liver or mammary gland cytosol. Chem Res Toxicol 15:536–544.

Sattsangi P, Leonard N, Frihart R. 1977. 1,N^2-Ethenoguanineand N^2,3-ethenoguanine: Synthesis and comparison of the electronic spectral properties of these linear and angular triheterocycles related to the Y bases. J Org Chem 42:3292–3296.

Tang N, Hattori T, Taga R, Igarashi K, Yang X, Tamura K, Kakitomo H, Mishukov V, Toriba A, Kizu R, Hayakawa K. 2005. Polycyclic aromatic hydrocarbons in urban air particulates and their relationship to emission sources in the Pan-Japan sea countries. Atmospheric Environ 39:5817–5826.

Vione D, Maurino V, Minero C, Pelizzetti E. 2005a. Nitration and photonitration of naphthalene in aqueous systems. Environ Sci Technol 39:1101–1110.

Vione D, Maurino V, Minero C, Pelizzetti E. 2005b. Aqueous atmospheric chemistry: Formation of 2,4-dinitrophenolupon nitration of 2-nitrophenol and 4-nitrophenol in solution. Environ Sci Technol 39:7921–7931.