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Rapid Report

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Elevated α-Methyl-γ-hydroxy-1,N^2-propano-2′-deoxyguanosine Levels in Urinary Samples from Individuals Exposed to Urban Air Pollution

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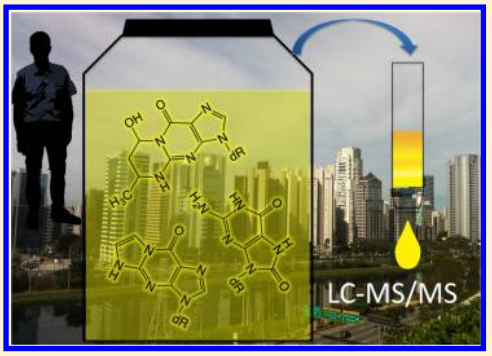
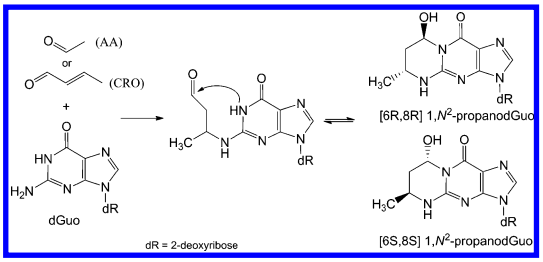
Supporting Information

ABSTRACT: Acetaldehyde and crotonaldehyde are genotoxic aldehydes present in tobacco smoke and vehicle exhaust. The reaction of these aldehydes with 2′-deoxyguanosine in DNA produces α-methyl-γ-hydroxy-1,N^2-propano-2′-deoxyguanosine (1,N^2-propanodGuo). Online HPLC-tandem mass spectrometry was utilized to accurately quantify 1,N^2-propanodGuo in human urinary samples from 47 residents of São Paulo City (SP) and 35 residents of the rural municipality of São João da Boa Vista (SJBV) in the state of São Paulo. Significantly higher 1,N^2-propanodGuo levels were found in the samples from SP donors than in samples from SJBV donors. Our results provide the first evidence that elevated levels of 1,N^2-propanodGuo in urinary samples may be correlated with urban air pollution.



Urban pollution in megacities, such as the São Paulo Metropolitan Area (SPMA), is produced by atmospheric emissions from vehicles and industries. Diesel, neat ethanol, and gasohol, a mixture of gasoline and anhydrous ethanol, are used as automotive fuels.[1] São Paulo City (SP) includes a fleet of approximately 7.4 million vehicles.[2] As a result, the SPMA features a specific atmospheric composition that differs from the atmospheric conditions of other megacities. De Martinis et al., investigated the mutagenicity of organic solvent extracts of PM_{10} (particulate matter with aerodynamic diameters of less than 10 μm) collected from SP. These researchers determined that the most mutagenic extract fractions contained aldehydes, ketones, carboxylic acids, and quinolines.[3] Acetaldehyde (AA) is widespread in the environment, AA is not only present in cigarette smoke, foods, and beverages but also produced during ethanol metabolism and the combustion of alcohol and diesel fuels.[4] The International Agency for Research on Cancer has recently suggested AA as a high-priority agent for evaluation during the 2010−2014 time period.[5] Crotonaldehyde (CRO) is an important industrial chemical and environmental pollutant that is formed during the combustion of plant materials, including tobacco, and is present in mobile source emissions. CRO can be endogenously generated by the oxidative degradation of unsaturated lipids; in addition, it is a metabolite of *N*-nitrosopyrrolidine.[6] The formation of DNA adducts is regarded as a critical aspect of the toxicity mechanisms of AA and CRO.[7] AA primarily reacts with 2′-deoxyguanosine (dGuo) to form N^2-ethylidene-2′-deoxyguanosine. AA adduction in the resulting Schiff base DNA adduct leads to the formation of the (6S,8S) and (6R,8R) diasteroisomers of 1,N^2-propanodGuo[8] (Scheme 1). The unequivocal formation of these adducts in live cells treated with [$^{13}C_2$]-AA was recently demonstrated by our research group.[9] Moreover, 1,N^2-propanodGuo can also be derived from the direct reaction of CRO with dGuo in DNA.[10]

Scheme 1. Representation of the Formation of DNA Adducts from Acetaldehyde and Crotonaldehyde



Additionally, the reaction of epoxidized α,β-unsaturated aldehydes, which are end products of lipid peroxidation, with DNA generates ethano or etheno derivatives.[11,12] The use of noninvasive strategies for the detection and quantification of DNA adducts is an attractive approach for toxicological studies. At present, the most used marker for DNA damage generated by oxidative stress is 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo).[13]

In this study, online reverse-phase high-performance liquid chromatography (HPLC) separation with tandem mass spectrometry detection was utilized for the accurate quantification of 1,N^2-propanodGuo, 1,N^2-etheno-2′-deoxyguanosine (1,N^2-εdGuo), and 8-oxodGuo in human urinary samples

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collected from residents of a polluted city and an unpolluted region. The study subjects were all males and provided information regarding their age, weight, smoking habits, health problems, medication usage, dietary supplementation, and alcohol ingestion. From the study were excluded individuals who had consumed alcohol within the 3 days prior to urine collection; consumed amino acid-supplemented diets; experienced health problems; and were smokers (Table S1, Supporting Information). The work was approved by the Human Ethics Committee of the Institute of Biomedical Sciences of the University of São Paulo.

To 1.5 mL of collected urine cooled to 4 °C was added 1.5 mL of 5 mM ammonium acetate buffer, 0.1 mM deferoxamine mesylate (pH 5), 250 fmol of [$^{15}N_5$]-1,N^2-εdGuo, and [$^{15}N_5$]-1,N^2-propanodGuo. This solution was heated to 37 °C for 5 min and centrifuged for 15 min at 4000 rpm.[14] The urinary solution was then subjected to solid phase extraction (SPE) using a Strata C18E column (Phenomenex, Torrance, CA). The adducts were principally found in the 65:35 CH_3OH/H_2O mixture. The collected fractions were individually lyophilized and diluted in water (100 μL) for subsequent analysis (40 μL) by HPLC coupled with mass spectrometry (MS) using an API 4000 QTRAP mass spectrometer (Applied Biosystems). All MS parameters were adjusted to acquire the best $[M + H]^+/[M + H\text{-}2\text{-}D\text{-}erythro\text{-pentose}]^+$ transition. The adducts were detected by single reaction monitoring (SRM) of the following transitions in m/z values: 284→168 (8-oxodGuo), 292→176 (1,N^2-εdGuo), 297→181 ([$^{15}N_5$]1,N^2-εdGuo), 338→222 (1,N^2-propanodGuo), and 343→227 ([$^{15}N_5$] 1,N^2-propanodGuo). The study methodology was validated through evaluations of sensitivity, linearity, precision, accuracy, and adduct recovery (Supporting Information). Urine samples from 47 residents of SP and 35 residents of SJBV were analyzed. The creatinine concentration of each urinary sample was determined via a colorimetric procedure for adduct normalization, utilizing a kit produced by Labtest (Lagoa Santa, MG, Brazil). Figures 1 and S5 (Supporting Information) illustrate a representative HPLC/ESI-MS-MS chromatogram of one urinary sample after SPE elution. Significantly higher concentrations of 1,N^2-propanodGuo were present in urine samples from SP residents than in urine samples from SJBV residents, with median values of 20.8 and 7.9 fmol of 1,N^2-propanodGuo/mg creatinine, respectively (Figure 2). Levels of 1,N^2-εdGuo and 8-oxodGuo in the examined urine samples were also measured for the purposes of comparison; however, samples from SP and SJBV residents exhibited no significant differences in the concentrations of these two compounds, suggesting that the elevated 1,N^2-propanodGuo levels detected in samples from SP residents are derived from exogenous exposure (Table S1, Supporting Information). Age homogeneity from SJBV donors, in the experimental group cannot be discarded as a directional effect for selection bias or some specific age-related effect.

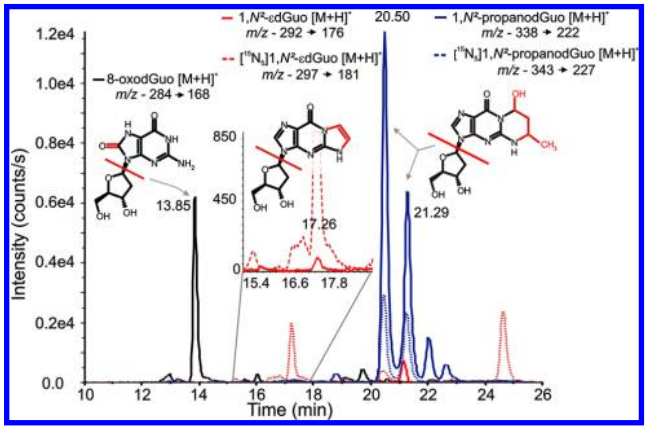

Figure 1. Representative chromatograms obtained from HPLC/ESI-MS/MS analyses of a fraction containing 2′-deoxyribonucleoside adducts. The chromatograms indicate the MS elution profile for 8-oxodGuo (13.85 min), 1,N^2-εdGuo, [$^{15}N_5$]1,N^2-εdGuo (17.26 min), 1,N^2-propanodGuo, and [$^{15}N_5$]1,N^2-propanodGuo diasteroisomers (20.50 and 21.29 min).

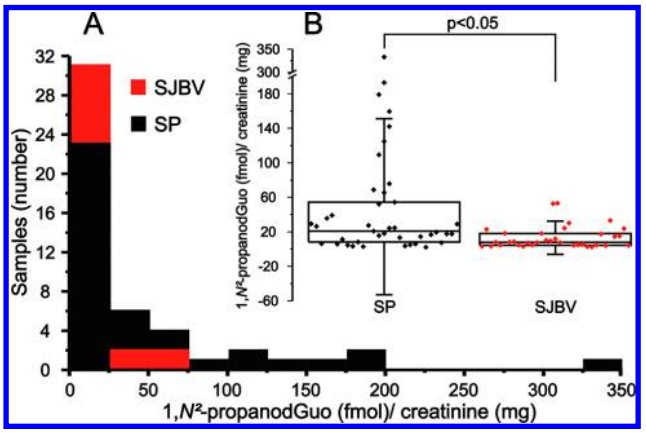

Figure 2. Global levels of two 1,N^2-propanodGuo diasteroisomers in urine samples from SP residents (polluted city, n = 47) and SJBV residents (unpolluted region, n = 35). (A) A histogram of 1,N^2-propanodGuo quantification results. Red bars indicate samples from SJBV residents, whereas black bars indicate samples from SP residents. (B) A boxplot for the SP and SJBV groups. $p < 0.05$ for the Kolmogorov−Smirnov and Mann−Whitney tests.

The presence of deoxynucleoside adducts in urine has been principally attributed to the occurrence of DNA repair, apoptosis, and reactions with deoxynucleoside pools.[15] More specifically, the presence of 8-oxodGuo in urine has been attributed to the degradation of 8-oxodGuo di- and triphosphate derivatives by the Nudix hydrolase family of enzymes.[16] It has been suggested that the levels of 8-oxodGuo in urine could serve as a potential biomarker of oxidatively generated DNA damage.[17] Base excision repair (BER) is the most important route for the removal of oxidative lesions in DNA, but some evidence indicates that nucleotide excision repair (NER) is also responsible for the repair of oxidized 2′-deoxyribonucleosides, such as 8-oxodGuo, which are subsequently excreted in urine.[18,19] Several mechanisms appear to be involved in the removal of exocyclic DNA adducts, including BER, NER, mismatch repair, and repair processes mediated by AlkB family proteins.[20,21] DNA adducts formed by CRO and AA are likely repaired primarily through homologous recombination and NER.[7,22]

Air pollution has been associated with adverse health effects and increased mortality among various age groups.[23] Elevated levels of oxidized forms of certain biomolecules, such as DNA and lipids, are strongly associated with exposure to particles produced by combustion, allowing these oxidized molecules to be used as biomarkers of biologically effective doses.[24]

1,N^2-PropanodGuo primarily promotes DNA miscoding in human cells through G→T transversions and can inhibit DNA replication.[25] Therefore, we conclude that urinary 1,N^2-

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propanodGuo levels could potentially be utilized as a suitable biomarker for monitoring AA/CRO exposure. These levels can be detected with high efficiency, specificity, and sensitivity using the HPLC/MS-MS methodology developed in this study. The monitoring of 1,N^2-propanodGuo levels in human urine may provide important information for the development of public policies for protecting the health of urban populations from the effects of air pollution.

■ ASSOCIATED CONTENT

Supporting Information

Detailed methodology and additional results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AA, acetaldehyde; CRO, crotonaldehyde; 8-oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 1,N^2-εdGuo, 1,N^2-etheno-2′-deoxyguanosine; 1,N^2-propanodGuo, α-methyl-γ-hydroxy-1,N^2-propano-2′-deoxyguanosine; dGuo, 2′-deoxyguanosine; SPE, solid phase extraction; HPLC/MS-MS, high-performance liquid chromatography/tandem mass spectrometry; BER, base excision repair; NER, nucleotide excision repair

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