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LC–MS/MS simultaneous quantitation of 2-hydroxyethylated, oxidative, and unmodified DNA nucleosides in DNA isolated from tissues of mice after exposure to ethylene oxide

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ABSTRACT

2-Hydroxyethylated and oxidative DNA nucleosides (DNA adduct biomarkers), such as O6-(2-hydroxyethyl)-2′-deoxyguanosine (O6HEdG), N6-(2-hydroxyethyl)-2′-deoxyadenosine (N6HEdA), 1-(2-hydroxyethyl)-2′-deoxyadenosine (N1HEdA), and 8-hydroxy-2′-deoxyguanosine (8-OHdG), N2,3-etheno-2′-deoxyguanosine (N2,3-ethenodG), α-methyl-γ-hydroxy-1,N2-propano-2′-deoxyguanosine (CrotondG), are important proposed biomarkers for exploring the genotoxicity mechanism of ethylene oxide (EO) in vivo. A liquid chromatography–tandem mass spectrometric method was developed for the simultaneous determination of O6HEdG, N6HEdA, N1HEdA, 8-OHdG, CrotondG, and N2,3-ethenodG together with regular 2′-deoxyguanosine (dG), and 2′-deoxyadenosine (dA) nucleosides in the DNA extracted from mouse lung tissues for the assessment of exposure to EO after inhalation. The lower limits of quantitation for 8-OHdG, CrotondG, N2,3-EthenodG, O6HEdG, N1HEdA, N6HEdA, dG, and dA were 0.025, 0.00125, 0.025, 0.00125, 0.025, 0.01, 2342, and 2500 ng/mL, respectively. The linearity of the calibration curves for all analytes were >0.989. The intra-day assay precision relative standard deviation (RSD) values for quality control (QC) samples for all analytes were ≤13.5% with accuracy values ranging from 86.5% to 111%. The inter-day assay precision (RSD) values for all analytes were ≤18.8% with accuracy values ranging from 87.9% to 119%. This method was used for simultaneous determination of the levels of 8-OHdG, CrotondG, N2,3-EthenodG, O6HEdG, dG, N1HEdA, N6HEdA, and dA in DNA enzymatic hydrolysates from DNA extracted from mouse lung after 12 weeks' inhalation exposure to EO at atmospheric concentrations of 0, 100, and 200 ppm. Overall, N2,3-ethenodG was not detected in any samples. 8-OHdG, CrotondG, dG, and dA were all quantifiable in all samples. O6HEdG, N1HEdA, and N6HEdA were quantifiable in most samples and the ratio of the corresponding adduct versus their corresponding DNA base (dG or dA) [×10 (e6)] was increased as the EO exposure concentration increased.

1. Introduction

Ethylene oxide (EO, oxirane, CASRN 75-21-8) is a highly reactive alkylating agent which is widely used in chemical synthesis and, to a minor degree, as a sterilant or fumigatant [1,2]. It is a colorless, flammable vapor at ambient temperature and pressure and is the simplest epoxide. Due to its wide usage, the toxic effects of ethylene oxide have been widely studied by several routes of administration for a variety of animal species [2]. Based primarily on animal data and cytogenetic changes in highly exposed workers, the International Agency for Research on Cancer (IRAC) classifies EO as a known human carcinogen [2]. For example, EO can induce alveolar/bronchiolar adenomas and carcinomas in the lungs of B6C3F$_1$ male mice at atmospheric concentrations of 50 and 10 ppm [3,4]. Previous work has identified a specific mutation in the K-*ras* oncogene that appears to be associated with the tumors [5]. The mode of action (MOA) of lung tumor formation is still not clear. However, based on the structure and known alkylating potential, EO is thought to induce mutations by reacting with cellular macromolecules to form covalent 2-hydroxyethylated adducts with both proteins and DNA [6–10]. These DNA adducts (especially guanine and adenine adducts) have been quantified in support of some of

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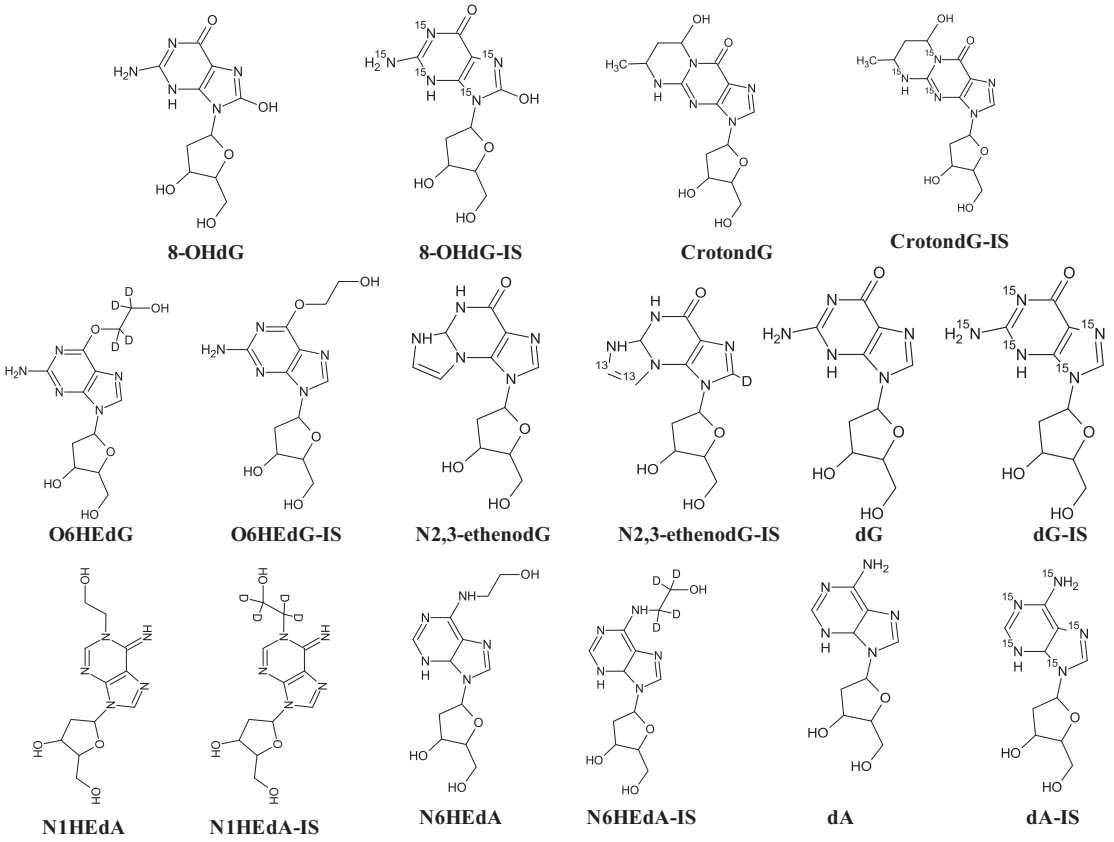

Fig. 1. Structures and abbreviations of analytes and internal standards.

the toxicity studies, including mutation and carcinogenicity studies [7–9,11]. In addition EO can also form 2-hydroxylated glutathione conjugates by direct alkylation of glutathione and deplete glutathione in both in vivo and in vitro conditions [12,13]. Glutathione depletion can potentially cause oxidative stress with subsequent formation of reactive oxygen species [14–16], which could lead to DNA oxidative damage as measured by biomarkers such as 8-OHdG, the most studied biomarker of oxidized DNA, as well as N2,3-EthenodG, and CrotondG the chosen biomarkers of lipid peroxidation formed by aldehydes generated from polyunsaturated fatty acid residues. If unrepaired, these DNA adducts may result in gene mutations [17–19]. Therefore, comprehensive analysis of both 2-hydroxyethylated and oxidative DNA nucleoside biomarkers is critical for exploring the MOA of genotoxicity caused by EO.

In recent years, various LC/MS–MS (liquid chromatography tandem mass spectrometry) methods for the detection and quantitative analysis of DNA nucleosides and DNA adducts have been published [20–23]. EO-derived DNA adducts have been also reported in conjunction with EO toxicity studies [12,24–26]. However, almost all published methods have focused predominantly on the major alkylation guanine adduct (N7-hydroxyethylguanine, N7-HEG). Recently, Tompkins et al. [1], developed a method for simultaneous detection of five different alkylation adducts (2-hydroxyethylated DNA adducts) using a high-performance liquid chromatography/electrospray ionization tandem mass spectrometry assay (LC/MS–MS). This method, however, did not provide information on oxidative DNA biomarkers such as 8-OHdG or CrotondG.

In order to efficiently explore the DNA adducts for genotoxicity MOA of EO in mice, we have developed and reported here a sensitive method of simultaneous quantitative analysis of both 2-hydroxyethylated and oxidative DNA nucleoside biomarkers, as well as unmodified DNA nucleosides using liquid chromatography-positive ionization electrospray tandem mass spectrometry (LC/ESI/MS–MS). This method was successfully used for simultaneous quantitation of 8-OHdG, CrotondG, O6HEdG, N2,3-EthenodG, dG, N1HEdA, N6HEdA, and dA in DNA samples extracted from mouse lung after 12-weeks' inhalation exposure to atmospheric EO concentrations of 0, 100, and 200 ppm.

2. Materials and methods

2.1. Caution

2.1.1. Chemical hazards

Ethylene oxide was handled in accordance with NIH guidelines for Laboratory Use of Chemical Carcinogens [27].

2.2. Reagents, solvents and materials

Acetonitrile (HPLC grade), methanol (HPLC grade), water (HPLC grade), acetic acid (HPLC grade), and ammonium acetate were obtained from Fisher Scientific (Itasca, IL, USA). DNase I, phosphodiesterase I, and alkaline phosphatase were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were also purchased from Sigma–Aldrich (St. Louis, MO, USA).

8-OHdG, dG, and dA (Fig. 1) were purchased from Sigma–Aldrich (St. Louis, MO, USA). 8-OHdG-IS, dG-IS, and dA-IS (Fig. 1) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). O6HEdG, O6HEdG-IS, CrotondG, CrotondG-IS, N1HEdA, N1HEdA-IS, N6HEdA, N6HEdA-IS, N2,3-EthnodG, and N2,3-EthenodG-IS (Fig. 1) were purchased from Toronto Research Chemicals Inc. (North York, Ontario, Canada).