Inhalation Exposure of Rats and Mice to 1,3-Butadiene Induces N⁶-Adenine Adducts of Epoxybutene Detected by ³²P-Postlabeling and HPLC

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In this paper we report DNA binding of butadiene monoxo, a first metabolite of 1,3-butadiene catalyzed by monoxygenases. We prepared alkylated purines as marker compounds for ³²P-postlabeling and electrochemical analysis and developed methods to measure the corresponding products. The traditional postlabeling assay was modified by incorporating a solid phase extraction column and high-performance liquid chromatography (HPLC) enrichment steps to the assay prior to labeling. The final analysis of adducted N⁶ adenines is based on two dimensional thin-layer chromatography (TLC) and an on-line HPLC radioactivity analysis. The qualitative and quantitative results are based on positively identified marker compounds. Alkylated N⁶ guanines were released from DNA by neutral thermal hydrolysis, prepared by HPLC, and analyzed by HPLC with a sensitive electrochemical detection procedure. By using these methods, we found alkylation of calf thymus DNA exposed to butadiene monoxo in vitro at adenine N⁶ and guanine N⁷ sites. Analysis of lung DNA samples from mice and rats exposed to butadiene through inhalation showed that adenine N⁶ adducts were formed in vivo in a dose responsive manner. — Environ Health Perspect 104(Suppl 3):655–657 (1996)

Key words: 1,3-butadiene, 3,4-epoxy-1-butene, inhalation, DNA, adducts, ³²P-postlabeling, adenine, guanine, HPLC

Introduction

1,3-Butadiene (BD) is not only a high production volume chemical for the polymer industry but is also a common environmental contaminant. It has been estimated that most environmental BD emissions derive from mobile sources. Burning of organic material produces emissions containing low concentrations of BD. Exposure to low concentrations of BD is thus a common characteristic of the entire human population (1).

Long-term inhalation studies indicate that BD is a multisite carcinogen in mice and rats, mice being by far the more sensitive species (2). A recent epidemiological study in the BD industry indicates an elevated mortality of lymphatic and reticular sarcoma among the production workers (3).

Small alkylating agents react mainly with the N7 position of guanine, but other alkylation sites in DNA are also detected in vitro (4,5). Goodrow et. al (6) have reported activation of the K-ras protoonogene at codon 13 involving a mutation in guanine. Butadiene monoxo (BMO), one of the reactive metabolites of BD, has been reported to alkylate guanine at the N7 position, but poor stability and low labeling efficiency of the product limits its use in postlabeling analyses (7,8). In an inhalation study in which transgenic mice were exposed to BD, an elevated level of A to T transversions were observed in the lacI gene (9). Although the alkylation at adenine may be a minor reaction site in DNA, this observation makes adenine an interesting alternative in DNA adduct monitoring.

In this paper we report alkylation of both adenine and guanine derived from BMO in relation to in vitro exposure to BMO or in vivo exposure to BD.

Materials and Methods

All commercial chemicals used in this study were of analytical grade. Adenine N⁶ adducts of BMO were prepared by reacting deoxyadenosine with 3’ and 5’-deoxadenosine monophosphates in alkaline conditions. The products formed were purified chromatographically and characterized by ultraviolet spectroscopy (UV), nuclear magnetic resonance (NMR), and mass spectrometry (MS) (10,11). Guanine N⁷ adducts were prepared and analyzed as described by Neagu et al. (7). The structures of the compounds studied are shown in Figure 1.

Calf thymus DNA (1 mg/ml) was exposed to BMO (10 µl/ml) for 1 and 6 hr. BMO was evaporated from the samples and the samples were subjected to enzyme-mediated digestion or thermal hydrolysis.

Rats and mice were exposed to BD for 5 days, 6 hr/day (50, 200, 500 ppm). DNA for N⁶ adduct analysis was isolated using a phenol extraction procedure (12). For the postlabeling analysis, an aliquot of DNA was taken and digested to 3’ nucleotides with micrococcal nuclease and spleen phosphodiesterase. BMO alkylated 2’-deoxyadenosine-3’-monophosphates were first enriched with disposable solid phase extraction cartridges (octadecylsilane phase). Normal nucleotides (NNs) were washed out from the column with 50 mM ammonium formate (AF) containing 3% methanol, pH 4. The amount of DNA

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digested was measured from the normal nucleotides by HPLC. N\textsuperscript{6} alkylated adenine adducts were eluted with 50% water/methanol.

Adduct enrichment was performed with reversed-phase HPLC. A fraction was collected from 17 to 20 min (residual NNs had the following retention times: 2'-deoxyctydine-3'-monophosphate, 4.3 min; 2'-deoxyguanosine-3'-monophosphate, 5.0 min; 2'-deoxymethylidine-3'-monophosphate, 6.6 min; 2'-deoxyadenosine-3'-monophosphate, 7.7 min). Enriched adducts were postlabeled by T4 kinase; the formed bisphosphates were treated with nuclease P1 resulting in 5' monophosphate adducts. The samples were spiked with the corresponding 5' compound and separated by two dimensional TLC chromatography. Dimension 1 was 0.3 M AF and dimension 2 was done with isobutryric acid/ammonia/water eluent in a ratio 69/1/30.

The adducts were located on TLC plates with autoradiography (Figure 2); the spot was scraped and consequently extracted with 1M AF. The ultimate analysis was carried out on a reversed-phase HPLC equipped with UV and radioactivity detectors. The HPLC gradient was 3% acetonitrile maintained for 5 min followed by a linear gradient to 16% during 26 min. The buffer was 0.75M AF and the ratio of scintillation solvent to HPLC effluent was 4:1.

**Results and Discussion**

We applied a solid phase extraction (SPE) procedure as a first step in our analytical protocol. The alkylated N\textsuperscript{6} products were retained in the cartridge and the normal nucleotides were washed out. The SPE step was necessary to avoid column loading caused by NNs in preparative HPLC. If severe column loading occurs, NNs may interfere with the postlabeling analysis.

By carrying out an ion exchange TLC and a reversed-phase HPLC, the analysis used two different retention mechanisms, which improved the qualitative reliability of the analysis. From a quantitative point of view, the use of a HPLC/radioactivity detector decreases the detection limit by the factor of 3 when compared to traditional scintillation counting. However, the standard deviation is much less pronounced if the analysis is carried out using the HPLC/radioactivity detector (SD ± 4%; n = 3) instead of the commonly used Cherenkov counting technique (SD ± 23%; n = 3).

![Figure 1](image1.png)

**Figure 1.** Structures of butadiene monoxide purine adducts studied. C1 and C2 refer to the carbon involved in the covalent bond formation.

![Figure 2](image2.png)

**Figure 2.** Two dimensional TLC (A) and a relative map position (B) of normal nucleotides to N\textsuperscript{6} alkylated 2'-deoxyadenosine-3'-monophosphate. Abbreviations: dCMP, 2'-deoxyctydine-3'-monophosphate; dGMP, 2'-deoxyguanosine-3'-monophosphate; dTMP, 2'-deoxymethylidine-3'-monophosphate; dAMP, 2'-deoxyadenosine-3'-monophosphate.

When calf thymus DNA was exposed to BMO for 1 and 6 hr, five times more adducts (both N7 and N6) were observed with the longer time exposure. The amount of N7 alkylated guanines was about 300 times higher than the amount of N6 products; however, the stability of the product was shown to be very different. As reported by Neagu et. al. (7), the N7 products showed a half life of 48 hr, but the N6...
products were stable and showed no decrease in the adduct levels within the 2-week period studied. This fact may favor the use of \( N^6 \) adducts in future biomonitoring applications because the \( N^6 \) adducts of adenine are able to reflect cumulative effects from long-term exposure.

Two dimensional TLC by itself was not able to resolve all of the interfering products present in the \( \text{in vivo} \) samples. By combining a subsequent HPLC analysis with a radioactivity detector, the selectivity of the analysis was much improved (Figure 3). In the lung samples of the rats and mice exposed by inhalation to BD (Figure 4), a dose–response increase of the \( N^6 \) adenine alkylation by BMO was detected. Insignificant differences were seen between the species, the lung tissue of mouse being only very weakly more sensitive than the rat lung to the BMO-derived \( N^6 \) adenine adduct formation. At the lowest exposure level (50 ppm) no differences were seen in the adduct levels when compared to the control animals in both species. This is mainly due to the nonspecific radioactivity detected close to the monitored product.

Work is ongoing to further lower the background level observed in the \( \text{in vivo} \) samples. We are also in the process of analyzing the \( N^2 \) adducts of guanine in the corresponding lung samples from which the \( N^6 \) adducts of adenine are now reported.

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Figure 3. HPLC chromatogram of a mouse lung sample. The signal was measured on-line as counts using a radioactivity detector. The arrow indicates the \( N^6 \) alkylation product.

Figure 4. The amount of \( N^6 \) alkylated adenines found in lung samples of mice and rats exposed through inhalation to 1,3-butadiene at various concentrations. dAMP, 2'-deoxyadenosine-3'-monophosphate. (Three animals of each species per concentration were analyzed in triplicate.)