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Advances in Brief

Analysis of Diepoxide-Specific Cyclic *N*-Terminal Globin Adducts in Mice and Rats after Inhalation Exposure to 1,3-Butadiene

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Abstract

1,3-Butadiene is an important industrial chemical used in the production of synthetic rubber and is also found in gasoline and combustion products. It is a multispecies, multisite carcinogen in rodents, with mice being the most sensitive species. 1,3-Butadiene is metabolized to several epoxides that form DNA and protein adducts. Previous analysis of 1,2,3-trihydroxybutyl-valine globin adducts suggested that most adducts resulted from 3-butene-1,2-diol metabolism to 3,4-epoxy-1,2-butanediol, rather than from 1,2;3,4-diepoxybutane. To specifically examine metabolism of 1,3-butadiene to 1,2;3,4-diepoxybutane, the formation of the 1,2;3,4-diepoxybutane–specific adduct *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)-valine was evaluated in mice treated with 3, 62.5, or 1250 ppm 1,3-butadiene for 10 days and rats exposed to 3 or 62.5 ppm 1,3-butadiene for 10 days, or to 1000 ppm 1,3-butadiene for 90 days, using a newly developed immunoaffinity liquid chromatography tandem mass spectrometry assay. In addition, 2-hydroxy-3-butenyl-valine and 1,2,3-trihydroxybutyl-valine adducts were determined. The analyses of several adducts derived from 1,3-butadiene metabolites provided new insight into species and exposure differences in 1,3-butadiene metabolism. Mice formed much higher amounts of *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine than rats. The formation of 2-hydroxy-3-butenyl-valine and *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine was similar in mice exposed to 3 or 62.5 ppm 1,3-butadiene, whereas 2-hydroxy-3-butenyl-valine was 3-fold higher at 1250 ppm. In both species, 1,2,3-trihydroxybutyl-valine adducts were much higher than 2-hydroxy-3-butenyl-valine and *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine. Together, these data show that 1,3-butadiene is primarily metabolized via the 3-butene-1,2-diol pathway, but that mice are much more efficient at forming 1,2;3,4-diepoxybutane than rats, particularly at low exposures. This assay should also be readily adaptable to molecular epidemiology studies on 1,3-butadiene-exposed workers

Introduction

1,3-Butadiene is an important industrial chemical used in the production of synthetic rubber and is also found in gasoline, cigarette smoke, and auto exhaust and as a product of combustion of wood and fossil fuels (1). It is a potent carcinogen in mice and a weak carcinogen in rats (1). Epidemiologic studies have shown an increased incidence of leukemia and lymphohematopoietic cancers in 1,3-butadiene–exposed workers (1). 1,3-Butadiene requires metabolic activation for its carcinogenicity (1). The major metabolites of 1,3-butadiene are 1,2-epoxy-3-butene, 3-butene-1,2-diol, 1,2;3,4-diepoxybutane, and 3,4-epoxy-1,2-butanediol (Fig. 1). To understand the mechanisms responsible for species differences in carcinogenic susceptibility, it is important to have an accurate measure of the internal doses of the different epoxides, because they exhibit up to 200-fold differences in their *in vitro* mutagenic potency, with 1,2;3,4-diepoxybutane being the most mutagenic (1). Protein adducts have been widely used to monitor the formation of alkylating metabolites. *In vitro* alkylation of valineamide or human hemoglobin with 1,2;3,4-diepoxybutane have demonstrated the preferential formation of the *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine adduct, suggesting that *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine is a specific marker for 1,2;3,4-diepoxybutane (2–4). Törnqvist *et al*. (5, 6) first confirmed the *in vivo* formation of *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine in mice treated by intraperitoneal injection with high doses of 1,2-epoxy-3-butene or 1,2;3,4-diepoxybutane. In these studies, the *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine was determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis of the alkylated 7-mer peptide after tryptic digestion and high-performance liquid chromatography enrichment. Although this method could quantify *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine in globin from highly exposed mice, it lacked adequate sensitivity for the detection of *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)-valine in rats and was unsuitable for analysis of low-exposure samples. Our initial attempts to measure *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine based on acid hydrolysis of globin followed by high–performance liquid chromatography and/or chemical extraction, derivatization, and gas chromatography (GC)-MS/MS were unsuccessful. Analysis of tryptic digests of highly alkylated human globin by LC-MS/MS showed considerable promise but lacked adequate sensitivity (7). We herein report an improved method that is capable of detection of *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine in rats and mice exposed to concentrations as low as 3 ppm 1,3-butadiene by inhalation. This method should also be amenable to the analysis of human globin samples.

Materials and Methods

Materials. Trypsin (biotin-agarose, from bovine pancreas) was purchased from Sigma-Aldrich (St. Louis, MO). All other reagents and solvents were ACS grade or higher. Centricon 3 filters were obtained from Amicon, Inc. (Beverly, MA), and Microspin filter tubes (regenerated cellulose, 0.2 μm) were from Alltech Associates, Inc. (Deerfield, IL). Red blood cells from untreated mice and rats were from PelFreez Biologicals (Rogers, AR).

Synthesis of Peptide Standards. The *N*-terminal α-chain peptides for mouse (*pyr*-VLSGEDKSNIK), rat (*pyr*-VLSADDKTNIK), and human (*pyr*-VLSPADKTNVK) derived from *rac* 1,2;3,4-diepoxybutane were synthesized as described by Jayaraj *et al.* (8). For accurate quantitation, the corresponding stable isotope-labeled peptides were synthesized containing [$^2H_3$]L. The *pyr*-(1–7) peptide standards and internal standards were prepared from *pyr*-(1–11) peptides by trypsin hydrolysis. In addition to the previously described characterization (8), all standards were sequenced by LC-MS/MS on an LCQ-Deca ion trap mass analyzer (ThermoFinnigan, San Jose, CA; data not shown).

Preparation of Immunoaffinity Columns. Polyclonal antibodies were raised against human *pyr*-(1–11) peptide, in which the C-terminal lysine was

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Fig. 1. Formation of *N*-terminal valine adducts from 1,3-butadiene.

replaced with a cysteine and conjugated to keyhole limpet hemocyanin (Anaspec, San Jose, CA). Four New Zealand White rabbits were immunized with the conjugate, and bleedings were withdrawn periodically. The sera were checked for *pyr*-(1–11) titer by enzyme-linked immunosorbent assay, and sera from the 2-month post-immunization bleed were selected to prepare immunoaffinity columns as described previously by Ham *et al.* (9).

Animals and Exposures. Globin samples were available from previous inhalation studies conducted at the Lovelace Respiratory Research Institute (Albuquerque, NM). Female B6C3F1 mice were exposed to 3, 62.5, or 1250 ppm 1,3-butadiene, 6 hours per day for 2 weeks (10 days); and female F344 rats were exposed to 3 or 62.5 ppm 1,3-butadiene for 10 days (10). In addition, globin samples were available from male and female Crl:CD rats exposed to 1000 ppm 1,3-butadiene for 90 days at the Haskell Laboratory for Toxicology and Industrial Medicine at DuPont (Newark, DE; ref. 11). All animals were sacrificed within 2 hours post-exposure by exsanguination under CO_2 anesthesia. Blood samples were collected by cardiac puncture. Red blood cells were isolated, washed twice with 0.9% saline, and stored at −80°C.

Globin Isolation and Trypsin Hydrolysis. Globin was precipitated and isolated according the procedure by Mowrer *et al.* (12). Globin samples (10 mg for mice and 30 mg for rats) were dissolved in 1 to 2 mL of 0.1 mol/L NH_4HCO_3 (pH 8), containing 2 pmol [$^2H_3$]*N*,*N*-(2,3-dihydroxy-1,4-butadiyl)-valine (1–11) peptide as internal standard. Ten μL of 10% SDS were added, and the globin was digested with 50 to 100 μL of trypsin-biotin agarose enzyme suspension at 37°C for 24 hours. Samples were filtered through Centricon-3 filters and dried by centrifugal lyophilization. For sample enrichment, samples were redissolved in 600 μL of PBS and loaded on immunoaffinity columns that had been preconditioned twice with PBS, water, 5% formic acid, water, and PBS. The columns were capped and left for 1 hour and washed five times with 7 mL of water. The alkylated 7-mer peptides were eluted in 5 mL of 5% formic acid and dried. Samples were reconstituted in 200 μL of water and filtered through Microspin filters (2 μm), dried, and stored at −20°C until analysis by LC-MS/MS.

Quantitative Analysis of 1,3-Butadiene-Derived Globin Adducts. The quantitative analysis of the *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine (1–7) peptides by capillary LC-electrospray-MS/MS was performed with an 1100 capillary high-performance liquid chromatograph (Agilent, Wilmington, DE) coupled to a TSQ-Quantum triple quad mass analyzer (ThermoFinnigan, San Jose, CA). A 0.3 × 150 mm Vydac C18 (238MS5), 5-μm column was operated with a linear gradient of 100% 15 mmol/L ammonium formate (pH 3.4) to 70% methanol-15 mmol/L ammonium formate (pH 3.4), in 10 minutes, at a flow rate of 7 μL/min. The *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine retention times were determined with *pyr*-(1–7) peptide standards. The peptides were detected in single reaction monitoring mode, monitoring the transition of the singly charged analyte (mouse and rat peptides *m/z* 833.5) and internal standard (mouse and rat peptides *m/z* 836.5) ions to the a_1-fragment (*m/z* 158.2). The MS conditions were as follows: spray voltage, 2200 V; heated capillary temperature, 350°C. The immunoaffinity purified samples were reconstituted in 10 μL of 15 mmol/L ammonium formate (pH 3.4), and depending on expected adduct levels, 1 to 8 μL (*e.g.,* 3 ppm 1,3-butadiene exposure) were injected with an 1100 capillary autosampler (Agilent).

Hydroxy-3-butenyl-valine and 1,2,3-trihydroxybutyl-valine adducts were determined according to a previously published GC-MS/MS method (13). In brief, alkylated *N*-terminal valine was selectively cleaved by Edman degradation using pentafluorophenyl isothiocyanate. [$^{13}C_5$]1,2,3-Trihydroxybutyl-valine or [$^{13}C_5$]2-hydroxy-3-butenyl-valine standard was added to the reaction mixture; and the pentafluorophenylthiohydantoin derivatives were extracted, acetylated (1,2,3-trihydroxybutyl-valine only), and analyzed by GC-MS/MS.

Results and Discussion

Determination of the molecular dose of 1,2;3,4-diepoxybutane in globin is critical for understanding the mechanisms of 1,3-butadiene carcinogenesis and mutagenesis, because 1,2;3,4-diepoxybutane is 100- and 200-fold more mutagenic *in vitro* than 1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol, respectively (1). Although the analyses of 2-hydroxy-3-butenyl-valine and 1,2,3-trihydroxybutyl-valine have greatly enhanced our knowledge on 1,3-butadiene metabolism across species, the extent of formation of 1,2,3-trihydroxybutyl-valine from 1,2;3,4-diepoxybutane *versus* 3,4-epoxy-1,2-butanediol could not be fully distinguished (7, 14). This report describes the first analysis of 1,2;3,4-diepoxybutane-specific *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine adducts in mice and rats after 1,3-butadiene inhalation exposures at concentrations known to induce tumors in rodents and down to those approaching human occupational exposures.

This method is similar to the one previously reported by Törnqvist *et al*. (5, 6); however, only the combination of immunoaffinity chromatography and detection by capillary LC-MS/MS resulted in a robust method with sufficient sensitivity compared with previously published methods. To account accurately for recovery during sample work-up, including the tryptic digest, the 11-mer analytical and stable isotope-labeled internal peptide standards were synthesized for mouse, rat, and human α–chain globin (8). For sample enrichment, immunoaffinity chromatography, rather than high-performance liquid chromatography, was applied before analysis by capillary LC-MS/MS, to allow the analysis of larger amounts of globin.

Before the analysis of *in vivo* samples, the precision and accuracy of the method were determined using control globin from mice, rats, and humans spiked with the corresponding *pyr*-(1–11) peptide standards. The anti–human *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine antibody retained the *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine peptides of all three species with equal efficiency. The limits of detection ($S/N > 3$) were 10 fmol on column, and the limits of quantitation were 50 fmol on column. The coefficient of variation and accuracy were <10% (Table S1). The method was then used for the analysis of *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine in mice and rats exposed to 1,3-butadiene by inhalation. The 1,2;3,4-diepoxybutane–specific *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine adduct was detected at exposures as low as 3 ppm 1,3-butadiene in mice and rats (Fig. 2), demonstrating the suitability of the method for *in vivo* studies aimed at understanding the molecular mechanisms of 1,3-butadiene carcinogenesis. These data were compared with 2-hydroxy-3-butenyl-valine and 1,2,3-trihydroxybutyl-valine determined using previously developed GC-MS/MS methods (Table 1).

Despite the fact that limited samples from previous 1,3-butadiene exposure groups were available for analysis, several important observations were evident. Mice form much higher amounts of *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine than rats at the different exposures studied. Furthermore, the number of 2-hydroxy-3-butenyl-valine and *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine adducts in mice were exposure-related but similar to each other at 3 and 62.5 ppm 1,3-butadiene

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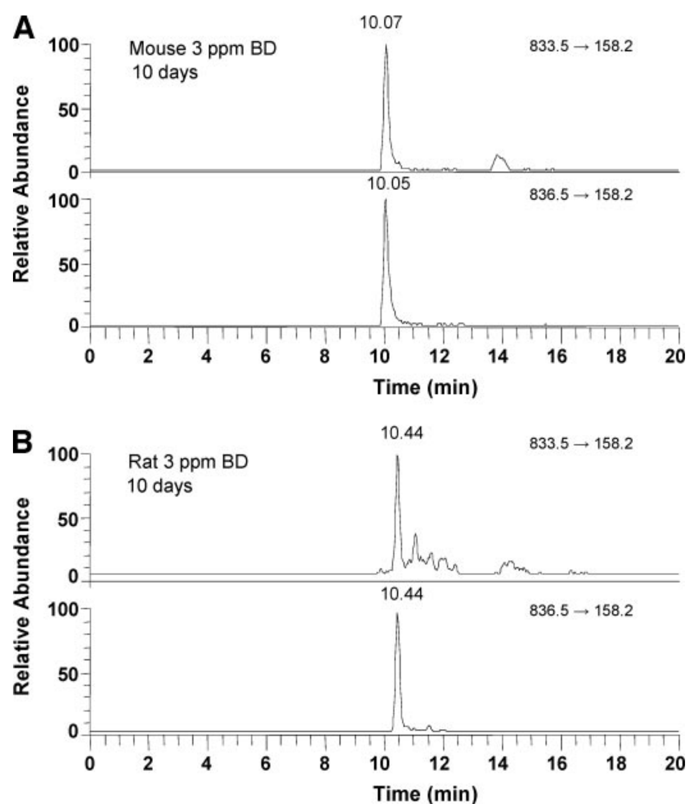

Fig. 2. Ion-chromatograms of *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine peptides from mouse (*A*) and rat (*B*) exposed to 3 ppm 1,3-butadiene for 10 days. Shown are the transitions of the analyte (833.5→158.2) and the internal standard peptides (836.5→158.2).

exposures, whereas 2-hydroxy-3-butenyl-valine was about 3-fold higher than *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine at 1250 ppm. These findings suggest more efficient metabolism of 1,2-epoxy-3-butene to 1,2;3,4-diepoxybutane at low exposures. In rats, the numbers of both adducts were lower than in mice, suggesting that the balance between epoxidation and hydrolysis favors hydrolysis in rats. In addition, 2-hydroxy-3-butenyl-valine was about 2.5-fold higher than *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine in rats, also indicating slower conversion of 1,2-epoxy-3-butene to 1,2;3,4-diepoxybutane, or more rapid hydrolysis of 1,2;3,4-diepoxybutane.

Surprisingly, the 1,2,3-trihydroxybutyl-valine values were similar in mice and rats at 3 and at 62.5 ppm 1,3-butadiene, and no consistent relationship between 1,2,3-trihydroxybutyl-valine and 2-hydroxy-3-butenyl-valine or *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine was observed at the different exposures studied. These phenomena are most likely due to the fact that 1,2,3-trihydroxybutyl-valine, as suggested previously, is mainly derived from the 3-butene-1,2-diol pathway (15–17). 3-Butene-1,2-diol, the precursor to 3,4-epoxy-1,2-butanediol, may also be converted to hydroxymethylvinyl ketone (18), that itself can form DNA (19) and *N*-terminal valine adducts (Dr. Mark Powley, personal communication). We are currently developing biomarker methods for the analysis of hydroxymethylvinyl ketone-derived DNA and protein adducts, which in combination with 1,2,3-trihydroxybutyl adducts will allow estimating the portion of 1,2-epoxy-3-butene that is metabolized via the 3-butene-1,2-diol pathway.

In rats, the formation of *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine (adducts in pmol/g per ppm 1,3-butadiene) was 12.5-fold lower than in mice at 3 ppm and 3.2-fold lower than in mice at 62.5 ppm. Comparisons between female rats exposed to 62.5 ppm 1,3-butadiene for 10 days *versus* 1000 ppm for 90 days demonstrated that the number of *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine adducts only doubled. This doubling most likely represents additional accumulation of adducts over the life span of erythrocytes (63 days), rather than an increase in 1,2;3,4-diepoxybutane formation at 1000 ppm 1,3-butadiene. These data suggest that in rats, the metabolism of 1,3-butadiene to 1,2;3,4-diepoxybutane is saturated at 62.5 ppm. Previous studies of 1,2,3-trihydroxybutyl-valine and 1,2,3-trihydroxybutyl-guanine in rats demonstrated saturation of 3,4-epoxy-1,2-butanediol formation (7, 16). In contrast, mice exhibited a decrease in slopes for 1,2,3-trihydroxybutyl-valine and 1,2,3-trihydroxybutyl-guanine between 62.5 and 1250 ppm 1,3-butadiene, but not saturation. Additional animal exposures are in progress that will provide samples to determine the extent of *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine formation in rats and mice of both sexes over a wide range of 1,3-butadiene exposures. From these ongoing studies, the rates of 1,3-butadiene uptake and the formation of the different metabolites and their respective globin adducts will be calculated and adjusted for species differences in life span of erythrocytes to allow a more exact comparison (6, 7) and to refine current physiologically based pharmacokinetic (PBPK) models (20).

In contrast to *in vitro* reactions, in which 1,2;3,4-diepoxybutane forms >99% *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine and only small amounts (<1%) of 1,2,3-trihydroxybutyl-valine (2), the amounts of 1,2,3-trihydroxybutyl-valine in both species of 1,3-butadiene exposed rodents were much higher (9.3–87-fold), than those for *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine. It was previously proposed that 1,2,3-trihydroxybutyl-valine adducts are mainly formed from 3,4-epoxy-1,2-butanediol and not from 1,2;3,4-diepoxybutane (15–17). The quantitative analysis of *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine

Table 1 *BD-derived globin adducts in mice and rats exposed for 10 days (n = 3) and rats exposed for 90 days (n = 6)*

Duration	Exposure (ppm BD)	HB-Val pmol/g	HB-Val adducts/ppm BD	*pyr*-Val pmol/g	*pyr*-Val adducts/ppm BD	THB-Val pmol/g	THB-Val adducts/ppm BD
10 days							
Mice (B6C3F1)							
	3	53 ± 7.6	17.7	48.7 ± 3.23	16.2	452 ± 38	150.0
	62.5	137 ± 12.2*	1.6	130.4 ± 64	2.1	3,410 ± 177	54.6
	1,250	7,143 ± 537	5.7	2,487.0 ± 426	2.0	13,755 ± 1,651	11.0
Rats (F344)							
	3	13 ± 2.4	4.3	3.9 ± 0.8	1.3	339 ± 41	113.0
	62.5	87 ± 7.6	1.4	38.3 ± 1.2	0.6	3,202 ± 302	51.2
90 days							
Rats (Crl:CD)							
Females	1,000	8,690 ± 930†	8.6‡	58.1 ± 17.3	0.058‡	24,066 ± 9,292	26.6‡
Males	1,000	5,480 ± 2,880†	5.4‡	16.7 ± 6.6	0.017‡	12,095 ± 3,712	12.7‡

Abbreviations: BD, 1,3-butadiene; HB-Val, 2-hydroxy-3-butenyl-valine; *pyr*-Val, *N,N*-(2,3-dihydroxy-1,4-butadiyl)–valine; THB-Val, 1,2,3-trihydroxybutyl-valine.
* One data point was excluded with 95% CI according to Q-test (*n* = 2).
† From ref. 13, *n* = 3.
‡ Efficiencies were based on 90-day exposures and were not adjusted per day of exposure.

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allows the first accurate estimation of the ratio between 3,4-epoxy-1,2-butanediol and 1,2;3,4-diepoxybutane formation. The ratios of 1,2,3-trihydroxybutyl-valine to *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine in mice exposed to 3 and 62.5 ppm 1,3-butadiene group were 9.3 and 29, respectively, and the corresponding ratios in rats were 87 and 83. These data support the hypothesis that 1,2-epoxy-3-butene is primarily metabolized via the 3-butene-1,2-diol pathway in both species. The results also demonstrate that mice, the most susceptible species, form considerably more 1,2;3,4-diepoxybutane than rats at the exposures studied.

Additional examination of the data for 2-hydroxy-3-butenyl-valine, *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine, and 1,2,3-trihydroxybutyl-valine as a whole clearly demonstrate that all of the epoxidation reactions proceed at their highest efficiency at low 1,3-butadiene exposures, with mice being more efficient in epoxide formation. The 1,2;3,4-diepoxybutane–specific adducts exhibit the greatest species difference, whereas 3,4-epoxy-1,2-butanediol adducts show the least difference. Previous studies measuring 1,2-epoxy-3-butene and 1,2;3,4-diepoxybutane concentrations in blood also demonstrated that mice were more efficient than rats in 1,2;3,4-diepoxybutane formation when exposed to 62.5 ppm 1,3-butadiene (21).

In summary, an immunoaffinity capillary LC-MS/MS method was established for the accurate measurement of the 1,2;3,4-diepoxybutane–specific *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine adduct, after exposures to 1,3-butadiene as low as 3 ppm in mice and rats. The optimization of this method represents a major advance in our ability to explore the effects of 1,3-butadiene exposure across species, including mice, rats, and humans, over exposure levels ranging from those encountered in occupational settings, to the high concentrations used in the earlier rodent studies. The use of immunoaffinity enrichment permits the detection of *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine in as little as 5 mg of globin from mice, as well as up to 200 mg of human globin (data not shown). Preliminary studies demonstrated clear species differences in the metabolism of 1,3-butadiene to 1,2;3,4-diepoxybutane that may be largely responsible for the differential susceptibility to 1,3-butadiene–induced carcinogenesis in rodents. Future comparative studies of 2-hydroxy-3-butenyl-valine, *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine, and 1,2,3-trihydroxybutyl-valine will provide more detailed knowledge of 1,3-butadiene metabolism across species and improve our understanding of the mechanisms of 1,3-butadiene–induced carcinogenesis. Analysis of *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)–valine in molecular epidemiology studies of 1,3-butadiene–exposed humans, combined with individual genetic polymorphism information, may make it possible to identify populations that are more or less susceptible to 1,3-butadiene–induced cancers and to develop more accurate cancer risk assessments.

Last, due to the availability of synthetic peptides and custom antibodies, this method may well be adapted for studies of other carcinogens or for quantitative analysis of other peptides (*e.g.*, other carcinogen modified *N*-terminal valine adducts or phosphorylated peptides such as map kinase proteins), making it a valuable tool for a variety of cancer researchers.

References

1. Himmelstein MW, Acquavella JF, Recio L, Medinsky MA, Bond JA. Toxicology and epidemiology of 1,3-butadiene. Crit Rev Toxicol 1997;27:1–108.
2. Rydberg P, Magnusson AL, Zorcec V, Granath F, Törnqvist M. Adducts to *N*-terminal valines in hemoglobin from butadiene metabolites. Chem Biol Interact 1996;101:193–205.
3. Basile A, Ferranti P, Pocsfalvi G, et al. A novel approach for identification and measurement of hemoglobin adducts with 1,2,3,4-diepoxybutane by liquid chromatography/electrospray ionisation mass spectrometry and matrix-assisted laser desorption/ionisation tandem mass spectrometry. Rapid Commun Mass Spectrom 2001;15: 527–40.
4. Miraglia N, Basile A, Pieri M, et al. Ion trap mass spectrometry in the structural analysis of haemoglobin peptides modified by epichlorohydrin and diepoxybutane. Rapid Commun Mass Spectrom 2002;16:840–7.
5. Kautiainen A, Fred C, Rydberg P, Törnqvist M. A liquid chromatography tandem mass spectrometric method for in vivo dose monitoring of diepoxybutane, a metabolite of butadiene. Rapid Commun Mass Spectrom 2000;14:1848–53.
6. Fred C, Kautiainen A, Athanassiadis I, Törnqvist M. Hemoglobin adduct levels in rat and mouse treated with 1,2:3,4-diepoxybutane. Chem Res Toxicol 2004;17:785–94.
7. Swenberg JA, Koc H, Upton PB, et al. Using DNA and hemoglobin adducts to improve the risk assessment of butadiene. Chem Biol Interact 2001;135–136:387–403.
8. Jayaraj K, Georgieva NI, Gold A, et al. Synthesis and characterization of peptides containing a cyclic Val adduct of diepoxybutane, a possible biomarker of human exposure to butadiene. Chem Res Toxicol 2003;16:637–43.
9. Ham AJL, Ranasinghe A, Morinello EJ, et al. Immunoaffinity/gas chromatography/high-resolution mass spectrometry method for the detection of N-2,3-ethenoguanine. Chem Res Toxicol 1999;12:1240–6.
10. Meng Q, Henderson RF, Long L, et al. Mutagenicity at the Hprt locus in T cells of female mice following inhalation exposures to low levels of 1,3-butadiene. Chem Biol Interact 2001;135–136:343–61.
11. Bevan C, Stadler JC, Elliott GS, et al. Subchronic toxicity of 4-vinylcyclohexene in rats and mice by inhalation exposure. Fund Appl Toxicol 1996;32:1–10.
12. Mowrer J, Törnqvist M, Jensen S, Ehrenberg L. Modified Edman degradation applied to hemoglobin for monitoring occupational exposure to alkylating-agents. Tox Env Chem 1986;11:215–31.
13. Swenberg JA, Christova-Gueorguieva NI, Upton PB, et al. 1,3-Butadiene: cancer, mutations, and adducts. Part V. Hemoglobin adducts as biomarkers of butadiene exposure and metabolism. Health Effects Institute Report 92. Cambridge, MA: HEI; 2000. p. 191–209.
14. Albertini R, Clewell H, Himmelstein MW, et al. The use of non-tumor data in cancer risk assessment: reflections on butadiene, vinyl chloride, and benzene. Regul Toxicol Pharmacol 2003;37:105–32.
15. Perez HL, Lahdetie J, Landin H, et al. Haemoglobin adducts of epoxybutanediol from exposure to 1,3-butadiene or butadiene epoxides. Chem Biol Interact 1997;105: 181–98.
16. Koc H, Tretyakova NY, Walker VE, Henderson RF, Swenberg JA. Molecular dosimetry of *N*-7 guanine adduct formation in mice and rats exposed to 1,3-butadiene. Chem Res Toxicol 1999;12:566–74.
17. Koivisto P, Kilpelainen I, Rasanen I, Adler ID, Pacchierotti F, Peltonen K. Butadiene diolepoxide- and diepoxybutane-derived DNA adducts at *N*7-guanine: a high occurrence of diolepoxide-derived adducts in mouse lung after 1,3-butadiene exposure. Carcinogenesis (Lond) 1999;20:1253–9.
18. Krause RJ, Kemper RA, Elfarra AA. Hydroxymethylvinyl ketone: a reactive Michael acceptor formed by the oxidation of 3-butene-1,2-diol by cDNA-expressed human cytochrome P450s and mouse, rat, and human liver microsomes. Chem Res Toxicol 2001;14:1590–5.
19. Powley MW, Jayaraj K, Gold A, Ball LM, Swenberg JA. 1,N^2-propanodeoxyguanosine adducts of the 1,3-butadiene metabolite, hydroxymethylvinyl ketone. Chem Res Toxicol 2003;16:1448–54.
20. Johanson G, Filser JG. PBPK model for butadiene metabolism to epoxides: quantitative species differences in metabolism. Toxicology 1996;113:40–7.
21. Henderson RF, Thornton-Manning JR, Bechtold WE, Dahl AR. Metabolism of 1,3-butadiene: species differences. Toxicology 1996;113:17–22.

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