A new sensitive determination method of propylene oxide-hemoglobin adducts by EI-GC-MS (SIM)

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Abstract

A gas chromatography/mass spectrometric assay method was developed for the determination of propylene oxide adduct, N-(3-hydroxypropyl)valine. Adduct was released from hemoglobin by alkaline hydrolysis and extract at pH 8 with ethyl ether. The dried extract was completely derivatized with N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA)/TMS-I (100:3). The detection limits of the assay were 0.08 ng/g for N-(3-hydroxypropyl)valine based upon assayed hemoglobin of 0.1 g. The method was applied to the determination of propylene oxide adduct formed in young female Sprague-Dawley rats after treatment for 1, 2 and 3 weeks with 0.008 % propylene oxide via the drinking water. An adduct was detected by proposed procedure. The structure of the adduct could be assigned to N-(3-hydroxypropyl)valine.

Introduction

Propylene oxide (PO, methyloxirane; CAS 75-56-9) is important industrial chemical that is produced mainly for chemical synthesis such as the production of glycols and glycol ethers. PO is carcinogenic to rodents as shown in the studies of the United States National Toxicology Program (NTP 1985).

Estimation of exposure to PO by air monitoring is complicated if the exposure is variable and occurs intermittently. Moreover, air-monitoring data refer to potential exposure rather than to the actual dose received by the exposed operators. These problems can be solved by the determination of biomarkers such as adducts to proteins or DNA. Adduct to Hb is especially suitable because small molecule such as PO form chemically stable adduct with the N-terminal valin of Hb, N-(3-hydroxypropyl)valine.

Chromatographic methods have been published for the analysis of PO adduct, N-(3-hydroxypropyl)valine [Boogaard 1999, Pauwels 1998]. Mass spectrometry has the potential to become a standard analytical tool for detecting N-(3-hydroxypropyl)valine, and sensitive analysis of N-(3-hydroxypropyl)valine has been usually accomplished by gas chromatography/mass spectrometry (GC/MS) [Boogaard 1999, Pauwels 1998]. Until now, all researcher have described a GC-MS method to determine the compound by converting N-(3-hydroxypropyl)valine to their pentafluorphenylthiohydantoin derivative. But the method is too complex and time consuming.

This paper describes the complete silylation method of N-(3-hydroxypropyl)valine with MSTFA/TMS-I (100:3). The method was applied to the determination of PO-hemoglobin-adduct formed in rats.

Materials and Method

Chemicals and reagents : PO, trimethylsilyl imidazole (TMS-I) and N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) were purchased from Sigma (St. Louis, MO, USA). Analytical grade of potassium carbonate, potassium hydroxide, potassium bishydrogen phosphate, sodium sulfate, hydrochloric acid and sodium chloride (Sigma, St. Louis, MO, USA) were used as reagents and ethyl ether, methanol, ethanol, acetone and ethyl acetate (E. Merck, Darmstadt, Germany) were used as solvents.

Animals and treatment : Twenty young female Sprague-Dawley rats with a body weight of about 220 g were obtained from Haehanbiolink (Chongju, Korea). They were acclimatized for one week in Macrolone cages (temp. of 18 ℃, humidity of 30~70 %, illumination time from 6 a.m. to 6 p.m.) and they had free access to tap water and food. The animals (3 per group) were treated with PO in the drinking water for 4 weeks at concentration of 0.008%. PO in the drinking water was prepared by dissolving 0.8g of PO in 10L of mineral water. After 1, 2 and 3 weeks of the treatment, the animals were killed by open heart puncture. Control animals were left untreated and killed after 3 weeks.

Isolation of hemoglobin : Erythrocytes were isolated from the blood by centrifugation and washed three times in phosphate-buffered saline Cells were lysed by adding four volumes of 0.1 mM EDTA, pH 7.5, at 35-41 °C in ultrasonic bath. Cell debris were removed by centrifugation (10,000 g). Hemoglobin was isolated by precipitation with acetonitrile and washed twice with acetonitrile/water, and finally with 80 % ethanol, 96 % ethanol, ethanol/diethyl ether and diethyl ether. Hemoglobin samples were dried and stored at 4 °C.

Hydrolysis of hemoglobin : 100 mg of dried hemoglobin was hydrolyzed for 3 h at room temperature with 3 mL of 0.1 M NaOH. N-(3-hydroxypropyl)valine released from hemoglobin was extracted with 7 mL of ethyl ether by mechanical shaking for 10 min. The organic phase was transferred into a 20 mL glass stoppered test tube and dried in evaporator and finally in a desiccator over P_2O_5/KOH for at least 30 min, before derivatization.

Derivatization : A dry residue is dissolved with 50 μL of MSTFA/TMS-I (100:3), and the tubes are heated at 80 °C. After 60 min, a 2 μL sample of the solution was injected in the GC system.

Gas chromatography-mass spectrometry : All mass spectra were obtained with a Agilent 6890/5973 N instrument. The ion source was operated in the electron ionization mode (EI; 70 eV, 230 °C). Full-scan mass spectra (m/z 40-800) were recorded for analyte identification. Separation was achieved with an HP fused-silica capillary column with cross-linked methylsilicone (HP 1), ~30 m length, 0.2 mm i.d., 0.33 μm film thickness. Samples were injected in the split mode with a splitting ratio of 1:8. The flow rate of the helium was 1.0 mL/min. The operating parameters were as follows: injector temperature, 280 °C; transfer line temperature, 300 °C; oven temperature, programmed from 80 °C at 15 °C/min to 300 °C (held for 5 min).

Results and Discussion

Derivatization : For the enhancement of GC performance (e.g. peak symmetry, resolution, peak height) and sensitivity of N-(3-hydroxypropyl)valine, derivatization of both hydroxy- and amino-group is necessary. In this derivatization condition, both hydroxy- and amino-group of N-(3-hydroxypropyl)valine were completely converted to a silylated derivative. The reaction rate of N-(3-hydroxypropyl)valine with MSTFA/TMS-I (100:3) was determined by the detection of the products. The derivatives were analyzed at reaction times of 5, 20, 40, 60 and 80 min. As a result, bis-TMS-N-(3-hydroxypropyl)valine was detected as a product of the silylation reaction of N-(3-hydroxypropyl)valine in about 60 min at 80 °C. The derivative was stable in chromatographic system and minimum for 1 week in reagents at room temperature.

Chromatography : The chromatogram of bis-TMS-N-(3-hydroxypropyl)valine derivative was obtained. For the GC separation of the derivatives, the use of the non-polar stationary phase was found to be efficient. The peak is symmetrical and no tailing can be seen. The drivative also do not show any adsorption effects in the GC system. The retention time of bis-TMS-N-(3-hydroxypropyl)valine were 9.15 min. There were no extraneous peaks observed in a chromatogram of blank biological sample at the retention time of the derivative.

Sensitivity : The combination of high derivatization yield and the high sensitivity of the derivative by EI-MS (SIM) permit their determination of hemoglobin-adduct at concentrations well below those reported previously. Detection limit was 0.08 ng/g for N-(3-hydroxypropyl)valine based upon an assayed hemoglobin of 0.1g. Limit was defined by a minimum signal-to-noise ratio of 3 and coefficients of variation for replicate determinations (n=5) of 15 % or less.

Application : After hydrolysis of hemoglobin samples isolated from PO-treated rats, extraction and derivatization, an adduct was detectable by GC-MS. The structure of these adduct could be assigned to N-(3-hydroxypropyl)valine by their retention times and fragmentation pattern obtained by GC-MS-EI. To assess the formation of the steady state of hemoglobin adducts during treatment with PO (0.008% PO in drinking water, 6.2 mg PO/kg body weight per day), blood samples from rats were taken 1, 2 and 3 weeks after initiation of treatment. After 1 week, the total mean amount of adducts determined was 3.5 ng/g hemoglobin. The adduct levels increased up to about 18.5 ng/g after week 1 and, thereafter, remained essentially constant.

Conclusion

The silylation derivative of N-(3-hydroxypropyl)valine with MSTFA/TMS-I (100:3) has good chromatographic property and offers very sensitive response for the EI-MS (SIM). The extraction of the compound from hemoglobin with ethyl ether also gave high recovery with small variation. Quantitation of N-(3-hydroxypropyl)valine is excellent, with linear calibration curves over a range of 1.0-100 ng/g and detection limit of 0.07 ng/g. The present method may also be applicable to the analysis of ethylene oxide-Hb adduct.

References

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