A study on Determination Method of (N-2-hydroxy-ethyl)valine(HEV) in Hemoglobin Adducts for Biological Monitoring of Ethylene Oxide Exposure

Jin Heon Lee, Ho Shang Shin and Hye Sil Ahn

Department of Environmental Education, Kongju National University, 182 Shinwhan, Gongju, Chungnam, 314-701, South Korea

Abstract

Ethylene oxide is a genotoxic carcinogen with widespread uses as industrial chemical intermediate and gaseous sterilant. 2-hydroxyethylated N-terminal valine in Hb is a good biomarker for biological monitoring of ethylene oxide exposure, because of its stability. We studied the determination method of (N-2-hydroxy-ethyl)valine in hemoglobin adduct by using GC/MS. PFPITC and TBMS were used as appropriate derivatives. Ethylene oxide formed Hb adducts as (N-2-hydroxy-ethyl)valine(HEV) in mouse with ethylene oxide inhalation exposure. Standard HEV can be synthesized with 2-amino-ethanol and 2-bromo-3-methylbutyric acid. GC/MS can measured them after derivatization with pentafluorophenylisothiocianate(PFPITC) and N-(tertiary butyl dimethylsilyl)-N-methyl-trifluoroacetamide(TBDMS-TFA) by using Edman procedure. Concentrations of Hb adduct were proportionally increased with exposure levels. They were 230±35(nmol g^{-1} globin) and 410±72(nmol g^{-1} globin) at 200ppm and 400ppm ethylene oxide inhalation exposure, respectively.

Introduction

Ethylene oxide is a genotoxic carcinogen with widespread uses as industrial chemical intermediate and gaseous sterilant(van Sittert, et. al., 1993; Walker, et. al., 1993; Fennell, et. al., 2000; Yong, et. al., 2001).

Adducts formed by reaction of chemicals and their metabolites in Hb provide a measures of assessing exposure and of measuring internal dose(Fennell, et. al., 2000). Adducts with N-terminal valine of Hb are chemically stable(Saha, et. al., 1995; Zhao & Hemminki, 2002). In contrast to most other proteins, such as serum albumin, which are subject to turnover with molecules being removed with apparent first order kinetics, Hb disappears from the circulation with apparent zero-order kinetics(Boogaard, 2002). These apparent zero-order kinetics are explained the stability of Hb in erythrocyte, which is not affected by the adduct of ethylene oxide with its N-terminal valine(Bolt, 1996; Boogaard, 2002). Hb is stable in erythrocytes and as

a consequence, its removal is entirely determined by the life-span of the erythrocytes. This means the exposure dose as measured by determination of Hb-adduct concentrations is integrated over the average life-span of human erythrocytes, which is approximately 4 months(Boogaard, 2002).

2-hydroxyethylated N-terminal valine in Hb adduct is a good biomarker for biological monitoring of ethylene oxide exposure(Ehrenberg, et. al., 1996; Bolt, et al., 1997; Carmella, et al., 2002). We studied the determination method of (N-2-hydroxy-ethyl)valine in hemoglobin adduct by using GC/MS. PFPITC and TBMS were used as appropriate derivatives.

Method

Materials: Ethylene oxide, 2-amino-ehtanol, 2-bromo-3-methylbutyric acid, MSTFA, NH_4I^+, MTBSTFA were obtained from Sigma(St. Louis, MO, USA). Analytical hydroxide, potassium bishydrogen phosphate, sodium sulfate, acetyl chloride and acetic ethanol, acetone and ethyl acetate(E. Merck, Darmstadt, Germany) were used as solvents. All other chemicals were of the highest purity available from Sigma and Merck.

Conditions of GC/MS: All mass spectra were obtained with 6890/5973 GC/MS(Agilent Techologies; Palo Alto, CA, USA). The ion source was operated in the electron ionization(EI) mode and the selected ion monitoring(SIM) mode(EI: 70 eV, 230℃). Full-scan mass spectra(m/z 30~800) were recorded for identification of analysts. Column was HP-5MS(30m×0.25mm× 0.25um F.T.). Samples were injected in the pulsed split ratio(1/10). The flow rate of helium was 1.0 ml/min. The GC operating temperatures were: injector temperature, 280℃; transfer line temperature, 280℃; oven temperature, programmed from 50℃ at 10℃/min to 300℃(hold for 5 min)(Lee et al., 2002).

Experimental schemes: Standard (N-2-hydroxy-ethyl)valine(HEV) was synthesized with 2-amino-ethanol and 2-bromo-3-methylbutyric acid. Its identification and measurement were done with various derivatives, such as pentafluorophenylisothiocian ate(PFPITC) and N-(tertiary butyl dimethylsilyl)-N-methyl-trifluoroacetamide (TBDMS-TFA). HEV was combined with derivatives by using N-alkyl Edman method(Tates, et. al., 1999). HEV levels in Hb adduct were measured in blood of mice with ethylene oxide inhalation exposure for 3 hr per day during 3 weeks.

Calculation: The dose, D, in a tissue is defined as the concentration-time integral with dimension mol kg^{-1}(or ℓ^{-1}) h = molar-hour(M h=1000mM h) of a reactive chemicals or metabolites (Ehrenberg, et al., 1983). With this definition, an adduct level, A(in mole g^{-1} globin), after acute exposure, is the product of D and the rate constant, k, for adduct formation. D is obtain from: D(mol kg^{-1} h) = A(mol g^{-1})/k(ℓ g^{-1} h^{-1}). The rate constant for reaction of ethylene oxide with terminal valine-N in mouse Hb has been determined to be: k_{val} = 5.8 x 10-5 ℓ (g globin)$^{-1}$ h^{-1}(Granath., et. al. 1999). The dose is related to the absorbed amount, C_0, of the chemical per kg body weight and the rate, $\lambda(h^{-1})$, of disappearance of the compound from the body: D(mol kg^{-1} h) = C_0 (mol kg^{-1})/$\lambda(h^{-1})$. Strictly, this relationship is valid if the dose is equally distributed in the body. An exposure chemicals may considered fully absorbed. From the equations above follows: A(mol g^{-1}) = $(C_0/\lambda)k_{val}$(Tates, et. al., 1999).

Results and discussion

(N-2-hydroxy-ethyl)valine(HEV): For synthesis of HEV, 1,440㎕ 2-amino-ethanol and 540㎖ 2-bromo-3-methylbutyric acid were dissolved with 360㎕ distilled water in 10㎖ test tube with cap, and kept overnight at 40℃. After adding acetone, a precipitate was purified with Dowex50 ion-exchange. It was used standard material for (N-2-hydroxy-ethyl)valine(HEV). For making 1000 ppm standard HEV solution, 10㎎ HEV was exactly dissolved with 10㎖ methanol. This solution was always stored at -20℃, dark space during the experimental period. Synthetic (N-2-hydroxy-ethyl)valine(HEV) was identified by using GC/MS with electron ionization(EI) mode. Its fragmentations were composed of m/z 130(M-31)+, m/z 118(M-43)+, m/z 116(base ion, M-45)+.

(N-2-hydroxy-ethyl)valine pentafluorophenylisothiocianate(HEV-PFPITC): For Synthesis of HEV-PFPITC, Synthetic HEV was dissolved with 40㎕ 1M NaOH, added 5㎕ pentafluorophenylisothiocianate(PFPITC), kept overnight at room temperature, and again reacted at 45℃ for 90 min. Synthetic HEV-PFPITC was extracted with toluene, washed with the distilled water and 0.1M Na_2CO_3 solution, condensed with nitrogen gas, and finally re-dissolved with toluene. Synthetic (N-2-hydroxy-ethyl)valine pentafluorophenylisothiocianate(HEV-PFPITC) was identified by using GC/MS with electron ionization(EI) mode. Its fragmentations were m/z 368(M)+, m/z 350(M-18)+, m/z 308(base ion, M-60)+.

(N-2-hydroxy-ethyl) valine pentafluorophenylisothiocianate-tertiary butyl dimethylsilyl(HEV-PFPITC-TBDMS): For synthesis of HEV-PFPITC-TBDMS, 50㎕ Synthetic HEV-PFPITC was reacted with TBDMSTFA:NH_4I(1000:3) at 80℃ for 1hr. It was used for HEV-PFPITC-TBDMS solution, and identified by using GC/MS with electron ionization (EI) mode. Its fragmentations were m/z 425(base ion, M-57)+.

Level of Hb adducts: Concentrations of Hb adduct were 0.04±0.003(nmol g^{-1} globin) at control, 230±35(nmol g^{-1} globin) at 200ppm ethylene oxide inhalation exposure group, and 410±72(nmol g^{-1} globin) at 400ppm exposure group.

Conclusion

Ethylene oxide formed Hb adduct as (N-2-hydroxy-ethyl)valine(HEV) in blood of mice with ethylene oxide exposure. It can be used biomarker for biological monitoring of ethylene oxide exposure, because of its stability. Standard HEV can be synthesized with 2-amino-ethanol and 2-bromo-3-methylbutyric acid. GC/MS can measured HEV after derivatization with pentafluorophenylisothiocianate(PFPITC) and N-(tertiary butyl dimethylsilyl)-N-methyl-trifluoroacetamide (TBDMS-TFA) by using Edman procedure. Concentrations of Hb adduct were proportionally increased with exposure levels. They were 230±35(nmol g^{-1} globin) and 410±72(nmol g^{-1} globin) at 200ppm and 400ppm ethylene oxide inhalation exposure, respectively.

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