Analysis of hydroxyethyl valine in hospital sterilization workers

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Received 16 March 2012; accepted 2 April 2012
Available online 11 May 2012

Abstract The formation of N-(2-hydroxyethyl) valine (HEV) in hemoglobin has been considered as a biologically effective dose for exposures to ethylene oxide (EO). In this study, 148 volunteers with no EO exposure history and 76 EO-exposed hospital sterilization workers in Taiwan were recruited, 10 ml of blood was collected, and background information was gathered using questionnaires from each study subject. HEV was processed by following the modified Edman degradation method and quantitated using a gas chirography coupled with mass spectrometry. Statistical analysis shows that the formation of HEV was significantly associated with smoking status and EO exposure.

Introduction

Ethylene oxide (EO) is widely used as a sterilant gas for heat-sensitive medical devices in hospitals, and it is also present in tobacco smoke (5 μg/cigarette) and the active metabolite converted from exogenous and endogenous ethylene. Hospital workers may be at risk of occupational EO exposures. Previous studies showed that EO exposures could cause reproductive toxicity in humans, such as increases in the risk of spontaneous abortion, preterm birth, and postterm birth among dental assistants exposed to EO. Several epidemiology studies reported significant increases in cancer risk among the EO-exposed workers. Currently, International Agency for Research on Cancer (IARC) classified EO as a human carcinogen. After uptake, EO can be detoxified by glutathione S-transferase theta or microsomal epoxide hydrolase in vivo. EO
can react with electrophilic sites of proteins to form the corresponding adducts. Analysis of protein adducts, particularly, hydroxethyl valine (HEV) in hemoglobin, can serve as biomarkers for cancer risk associated EO exposures and has been validated as a chemical-specific biomarker for the assessment of EO cumulative exposures in workplaces or other environments. 7–10

The objective of this study was designed to analyze HEV for the assessment of cumulative EO exposures for character-ization of host susceptibility among the EO-exposed hospital workers. These data will be exploited by statistical analysis to elucidate the interactive roles of EO exposures and will also be critical in future follow-up studies of health effects of low-dose EO exposures for these workers.

Materials and methods

Chemicals

High Performance Liquid Chromatography (HPLC) grade of water, isopropanol, and diethyl ether, ethyl acetate, hexane were bought from Tedia in the United States. Pentafluorobenzenethiol isothiocyanate was bought from Fluka (Buchs, Switzerland). 2H2-labeled EO was purchased from Cambridge Isotope (MA, USA). N2-hydroxyethylvaline-anilide was purchased from Bachem Biochemica (Germany). American Chemical Society (ACS) reagent of toluene and an ultra grade of formamide were purchased from Sigma (Germany).

Analysis of HEV using the modified Edman degradation method

A total of 76 sterilization workers in hospitals at Koao-suing City and County in Taiwan were recruited, and 148 volunteers without the history of EO exposure were randomly selected at a blood donation station in Taichung City, Taiwan. Each study participant was asked to answer questions in a survey that included items of sex, age, smoking habits (numbers of cigarette/per day), potential second-hand smoke exposure (due to smoking by family members or colleagues at the workplace), lifestyle (consumption of alcohol, number of cups of coffee, consumption of tea, and frequency of tea drinking). All samples were obtained with informed consent by following an Institutional Review Board protocol approved by National Health Research Institutes in Taiwan. A total of 5 µL of blood was collected from each study participant by ventipuncture using an ethylendiaminetetraacetic acid-coated syringe. Hemoglobin was extracted using the procedures previously described. Globin was derivatized by following the modified Edman degradation method for specific cleavage of N-alkylated terminal valines.11 HEV was quantitated by monitoring m/z 348 and 352. The amounts of HEV were calculated according to a calibration curve to a linear calibration curve with r = 0.9984.10

Statistical analysis

Mean and standard deviation were used to describe the distribution of age and HEV by the status of exposure. Smoking statuses were classified as a nonsmoker, and at least one-half a pack/day. Student’s t statistics were used to compare HEV exposure between sterilization workers in hospitals and volunteers without the history of occupa-tional EO exposure. The data were analyzed using the SAS statistical package. All of the p values were two-sided.

Results

The average ages for the volunteer group and the exposed workers are 32.0 and 39.4 years, respectively. Among the 148 volunteers, 78 (52%) were nonsmokers, and 31 (22.7%) smoked at least one-half a pack/day. The majority (88.2%) of EO-exposed workers were nonsmoker, and only nine of them were smokers.

The smoking and exposed study participants had higher amounts of HEV than the nonsmoking and nonexposed participants, respectively. The average HEV content was significantly higher in nonsmoking exposed workers (244 ± 350.4 p mol/g Hb) than that in nonsmoking volunteers (57.7 ± 45.6 p mol/g Hb). The smoking exposed workers had significantly higher HEV contents (542.2 ± 455.8 p mol/g Hb) than nonsmoking exposed workers and smoking volunteers (206.9 ± 148.6 p mol/g Hb). The greater variation of HEV levels in the workers than those in the volunteers was due to the greater variation of EO exposures among the workers.

Multiple-variable linear regression was performed for the combined data set of smoking volunteers and exposed workers. Smoking and exposure status were statistically significant (p < 0.001).

Discussion

All HEV contents in our study participants are less than 4000 p mol/g Hb, and this suggests that their EO exposures were less than 1 ppm. This indicates that all hospitals studied have complied with the current regulation of EO at 1 ppm in Taiwan. The cumulative EO exposures of the hospital steril-ization workers and smokers are about the same. These results are consistent with previous studies on EO-exposed workers in hospitals that most exposures occurred when handling the sterilization equipment EO exposure was negligible.12 Exposed workers have greater HEV levels than the smokers, suggesting that some of the workers might have been exposed to greater amounts of EO than the smokers.

The formation of HEV was predominated by EO exposures, and it was not affected by other factors such as age and lifestyle factors.10 Results from this study also confirm that EO exposures primarily determine the formation of HEV. The differences in HEV contents between exposed workers and smokers were determined by EO exposures as well as the differences between nonsmoking workers and smokers (or nonsmokers). Although the major limitation of this study has been the lack of personal sampling data, HEV contents can represents the cumulative EO exposures for the studied workers.

Acknowledgments

This study was supported by grant number EO-pp-92-07 from the Taiwan National Health Research Institutes.
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