行政院國家科學委員會補助專題研究計畫成果報告

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※氣相層析儀加質譜儀方法分析血紅素與環氧乙烷共價鍵結物作為生物有效劑量之研究※
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一、中文摘要

香煙中含有多種致癌物質，其中環氧乙烷的含量約為 5 μg/cig.，目前環氧乙烷已被歸類為人體致癌物，因此由於抽煙而暴露於環氧乙烷所造成人體之致癌風險是值得探討。本研究的目的係建立 modified Edman degradation 方法來測量環氧乙烷與血紅素形成之共價鍵結物 (N-hydroxyethylvaline；HEV)，以探討抽煙習慣與體內蛋白質鍵結物濃度的相關性，並由 HEV 濃度推估暴露到環氧乙烷之劑量及致癌風險。研究對象為台中捐血站之 150 位自願者，經問卷調查收集基本資料，及血液收集，分離紅血球並萃取血紅素，經處理後，利用 GC/MS-NCI 分析，以質荷比 348 與 352 之訊號比值來計算 HEV 之濃度。結果顯示非抽煙組體內之 HEV 濃度為 58.3±45.6 pmol/g Hb，抽煙組 HEV 濃度為 205.9±151.4 pmol/g Hb，國人 HEV 含量與文獻值一致，且抽煙與 HEV 濃度值達統計上顯著相關($p<0.0001$)，而每天抽煙支數及抽煙年數皆與體內 HEV 濃度達統計上顯著相關($p<0.0001$)。另外將 HEV 濃度值進行複迴歸分析，發覺抽煙與否、每天抽煙支數與體內 HEV 值有顯著相關。而非抽煙族群與抽煙族群致癌風險值平均分別為 4.15×10^{-5} 及 1.463×10^{-4}，兩者皆與抽煙達統計上顯著差異($p<0.0001$)。本研究結果證實體內 HEV 可作為評估長期因抽煙暴露到環氧乙烷之生物指標，且驗證長期抽煙會使癌症發生之危險性增加。

關鍵詞：N-(2-hydroxyethyl)valine、蛋白質鍵結物、環氧乙烷、GC/MS

Abstract

The formation of N-(2-hydroxyethyl) valine (HEV) in hemoglobin has been considered as a biologically-effective dose to assess ethylene oxide and ethylene exposures from tobacco smoke. The objective of this study was to specifically analyze HEV to study the association of life style with HEV formation. Blood (5 ml) was collected from each of 150 volunteers without history of occupational ethylene oxide or ethylene exposure at a blood donation station in Taichung, Taiwan. Questionnaires were used to gather individual information of consumption of tea, alcohol, and tobacco smoke. Samples were processed by following the modified Edman degradation method and quantitated using a Fission 8060 gas chromatograph coupled with a Platform mass spectrometer operated at negative chemical ionization mode. The amounts of HEV in smokers (204 ± 151 pmol HEV/g globin) were higher than nonsmokers (57 ± 46 pmol HEV/g globin) and increased with the numbers of cigarettes smoked per day with a rate of 8.8 pmol HEV/g globin/Cig/day, consistent with 16 - 58 pmol HEV/g globin in nonsmokers and 92 – 389 pmol HEV/g globin in smokers and fell between 7.1 to 11.0 pmol HEV /g globin /Cig/day in the literature. The associations of the formation of HEV with life styles were negative. The formation of HEV in study subjects were significantly affected by numbers of cigarette smoked ($P < 0.001$), years of cigarette smoking ($P < 0.001$), and consumption of tea among smokers ($P < 0.1$), but not by alcohol consumption, second-hand smoke, ages, or sex. These results suggest that the significant higher amounts of HEV in smokers than nonsmokers were mainly from exposures to EO or ET in cigarette smoke and probably in part due to the tobacco smoking resulting in lipid peroxidation which leads to the increase in the formation of HEV.

Keywords: N-(2-hydroxyethyl) valine,

smokers, nonsmokers, tea consumption

二、緣由與目的

Ethylene oxide (EO) is a known human carcinogen and a direct alkylating agent which attacks nucleophilic sites of biological molecules, such as proteins and DNA bases to form protein and DNA adducts (1-4). Among EO-induced protein adducts, N-terminal hydroxyethyl valine (HEV) of hemoglobin has been validated as a biologically effective dose in several animal species exposed to EO or ET (2-4). The measurements of HEV in humans have frequently been performed using a modified Edman degradation and gas chromatography (GC)/mass spectrometry (MS) to detect its pentafluorophenylthiohydantoin deterderivative (7). This assay was sensitive enough to quantitate background levels of HEV in nonsmokers without EO exposure history.

HEV values varied from 16 ± 7 to 58 ± 25 p mol/g globin in nonsmokers, and 92 ± 25 to 389 ± 138 p mol/g globin in smokers. Approximately 5 μg of EO estimated present in each cigarette smoked suggested that tobacco smoking is a cancer risk-associated factor. The amounts of HEV in nonsmokers could be contributed from endogenous and exogenous sources (4-6). Endogenous sources of EO found in unexposed animals and humans were mainly due to the metabolic product of ET produced from lipid peroxidation, oxidation of methionine and hemin, and metabolism of intestinal bacteria (5).

Therefore, the formation of HEV exhibits the summation effects of EO and ET exposures, lipid peroxidation, metabolism of ET, and detoxication of EO. Some of them have been investigated, such as the association of the formation of HEV with genetic polymorphism of glutathione S-transferase Theta (6). But, other factors, such as lifestyles, have not been systematically studied. The objective of this study was to use the modified Edman degradation method to analyze HEV in smokers and nonsmokers so as to evaluate the association between the formation of HEV and factors related to lifestyles.

三、結果

3.1. Background information of the study population

The background information of the study population is summarized. Total 148 volunteers without occupational EO exposure history were studied, their ages varied from 18-65 years old, 78 of them were nonsmokers, and 70 were smokers. 55.7% of smokers consumed 1 – 10 cigarettes per day, and 44.3% consumed 10 or more cigarettes per day. Also, 77.9% and 75% of smokers were exposed to second-hand smoke at home and at the workplace, respectively. Among the smokers, 33.7% consumed alcohol, 44.9 % drank tea, and 21.7% drank coffee. These numbers for nonsmokers were 15.7%, 42.7%, and 32.6%, respectively.

3.2. Quantitation of HEV in nonsmokers and a smokers

Samples were quantitated using GC/MS operated at NCI and selective ion mode (SIM). Mass/electron ratio at 348 and 352 were monitored, the ratio of peak area of HEV and $^{2}H_{4}$-labeled HEV and the amounts of HEV was used to construct the significant linear calibration curve. The amounts of HEV were calculated using this calibration curve.

The average amounts of HEV in smokers (N = 70) were 204 ± 151 pmol/g globin and significantly higher than 57 ± 46 pmol/g globin in nonsmokers (N = 78) in Taiwan. However, there was no significant difference in HEV content among male and female nonsmokers. The amounts of HEV increased with numbers of cigarettes smoked at a rate of 8.8 pmol HEV/g globin/day/cig with r = 0.53 when analyzed using simple linear regression. The amounts of HEV accumulated with the years of smoking, and increased with both pack of cigarettes x number of years smoking, and cigarettes smoked x number of years smoking. When analyzed using generalized linear model, the number of cigarettes smoked per day was the only factor that caused significant increases in amounts of HEV at P values less than 0.001. Age was not a significant factor. Furthermore, the amounts of HEV were stratified according to factors associated with lifestyles and analyzed using two-way ANOVA Results showed that sex and consumption of alcohol did not affect the formation of HEV among nonsmokers and smokers. Exposure to second-hand smokes at workplace was not significantly associated with the levels of HEV in smokers and nonsmokers.

4. 討論

Our results also showed smokers have higher amount of HEV (204.5 ± 151.0 pmol HEV/g globin) than nonsmokers (56.6 ± 45.6 pmol HEV/g globin). The increased rate in HEV, 8.8 pmol/g globin/Cig./day, also fell within the range from 7.1 to 11 pmol/g globin/Cig./day. Although these results are consistent with data in literatures, the amounts of HEV in nonsmokers are relatively high. Compared with estimated endogenous background HEV 12 ± 2.9 pmol/g globin (4), significant amounts of HEV in nonsmokers might be contributed by exogeneous sources of EO or ET probably due to busy traffic in Taichung, which is the third largest city in Taiwan with a population approximate at 1 million.

The amounts of HEV in smokers exposed to second-hand smoke at home (225 ± 160 pmol HEV/g globin) were significantly higher than in smokers who were not exposed to second-hand smoke at home (138.± 97 pmol HEV/g globin) with $P < 0.05$. This could be explained partly that smokers exposed to side-stream smoke at home (14.0 cigarettes/day) have smoke more cigarettes per day than smokers without exposures to second-hand smoke (9.2 cigarettes/day). Therefore, second-hand smoke might not have an impact on the formation of HEV.

Drinking tea has been a habit in a large proportion of Taiwanese. Consumption of tea did significantly reduce the formation of HEV at P less than 0.1. One third of the main components of tea is polyphenols, such as (-)-epigallocatechin gallate (EGCG), which is a potent antioxidant with free radical scavenging ability. Consumption of green tea and black tea was reported to increase total antioxidant content in human blood plasma. Green tea and black tea were found to reduce lipid peroxidation and oxidative DNA damage in smokers and nonsmokers by significantly reducing levels of malondialdeyhde and 8-hydroxy-deoxyguanosine (8). Our data showed that consumption of tea was weakly associated with the formation of HEV in smokers, but not in nonsmokers. However, smokers with tea consumption usually consumed more cigarettes than smokers with tea consumption.

In conclusion, the amounts of HEV in smokers were approximately 4-fold grater than those in nonsmokers. The differences was mainly due to exposures to EO and/or ET in tobacco smoke.

五、 計畫成果自評

本研究內容與原計劃相符程度高達85%以上，正如預期目標暴露 EO 的抽煙者其 HEV 含量比不抽煙者高，此技術的成功建立將可應用在許多的有害物質作長期暴露評估使用。本研究成果已投稿到 Mutation Research，因此在學術上與實際用上的價值都非常高。

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