附件：封面格式

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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※ Cyclopenta[cd]pyrene 在體內所引起 ※

※ 去氧核醣核酸傷害之探討 ※

※ Cyclopenta[cd]pyrene-Induced DNA Damage In Vivo ※

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計畫主持人：徐景宏

共同主持人：

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□赴國外出差或研習心得報告一份

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一、中文摘要

根據流行病學研究顯示空氣污染較嚴重地區居民之肺癌死亡率及發生率較高。而台灣都會區主要之空氣污染來自於機車排放廢氣中之致癌性多環芳香碳烴(PAHs)，例如 benzo[a]pyrene (B[a]P)、cyclopenta[cd]pyrene (CPP) 等，其總量遠超過其他國家。因此，了解 PAH 與肺癌發生間的關聯性，實為台灣環境醫學之重要課題。

本計劃針對 CPP 在體內試驗（動物模式）所產生之去氧核醣核酸鍵結物（DNA adducts）加以研究。目前成果包括 1)成功建立測量 CPP-DNA adducts 之 HPLC 條件及方法，2)已獲得 2 種主要 CPP-DNA 異構物標準品各約 1 mg [*cis*-3-(deoxyguaonsin-N2-yl)-4-hydroxy-3,4-dihydroCPP]，以及 3)探討影響正反 DNA adduct 異構物生成比例之催化因子（如鹵離子），期對於 CPP 與 DNA 間之化學鍵結機制能有較深入之瞭解。

關鍵詞：多環芳香碳烴、癌症、去氧核醣核酸鍵結物

Abstract

Air pollution is a serious human health problem around the world including Taiwan. Some genotoxic compounds have been found in airborne particles, including polycyclic aromatic hydrocarbons (PAHs). The amount of PAHs in air particles from urban area in Taiwan is much higher than that of UK, Japan, and US. Therefore, cancer, especially lung cancer, induced by PAHs should be an important environmental medical concern in Taiwan. Cyclopenta[cd]pyrene (CPP), a highly carcinogenic PAH, is a ubiquitous environment contaminant. It is usually found with benzo[a]pyrene (B[a]P) and from certain sources, it is up to 7-fold higher than B[a]P. Thus, it is chosen for this study.

The current project aims to investigate DNA modification caused by CPP (*in vivo* in the near future). We first developed the analytical method of CPP-DNA adducts using HPLC. Then, two major adducts, the diastereomers of *cis*-3-(deoxyguaonsin-N2-yl)-4-hydroxy-3,4-dihydroCPP), were made, with approx. 1 mg each. These synthetic standards will be employed for generating corresponding antibodies. We anticipate that an immunochemical method, or ELISA, for detecting CPP-DNA adducts will be established. ELISA is considered to be more suitable for adduct quantification when compared to the ^{32}P-postlabeling assay. To further understand the chemistry between CPP and DNA, factors that may affect the formation of different stereoisomeric adducts were examined.

Keywords: Polycyclic aromatic hydrocarbon, cyclopenta[cd]pyrene, cancer, DNA adducts

二、緣由與目的

Since 1982, malignant neoplasms have been the first leading cause of death in Taiwan. This figure has increased more than one-third since 1978, and is still increasing. Lung cancer is the leading cause of cancer death among women and the second cause of cancer deaths among men in Taiwan [1]. Although cigarette smoking is known to be

the major cause of lung cancer, other particular air pollutants may also contribute to the incidence of lung cancer, especially for Taiwanese women (the prevalence female smokers: 3.8%)(Cancer Registry Annual Report, ROC, 1992).

Exposure to chemical carcinogens is believed to be an important etiological factor in human cancer. Carcinogen-DNA adducts are considered to represent the initiating events leading to mutations and malignant transformation, which ultimately lead to cancer. The detection of DNA adducts and levels *in vivo*, therefore, can reveal DNA damage, or genotoxicity, and exposure to specific carcinogens. In fact, increased DNA adduct levels have been observed in human populations with known or suspected risk of developing cancer as well as in laboratory animals exposed to certain carcinogens.

Cheng et al. [2] reported that the total DNA adducts in 86 Taiwanese lung cancer patients were much higher than those of other countries, and there is no difference observed on DNA adduct levels between smoking and non-smoking lung cancer patients. Also, adduct levels of female non-smoking cancer patients were significantly higher than that of male non-smoking lung cancer patients. These results suggested that environmental air pollutants other than smoking are responsible for the formation of DNA adducts in lung tissue from lung cancer patients, especially for non-smoking patients, in Taiwan.

The major mutagenic and carcinogenic chemicals in airborne particulates include PAHs. The amounts of PAHs, such as B[a]P and CPP, in airborne particulate samples of Taiwan are greater than those from other countries [3]. Therefore, understanding PAH-induced cancer, especially lung cancer, should be an important environmental medical issue.

CPP, produced by incomplete combustion, is a widespread air contaminant. It is found in, for example, automobile exhaust [4], carbon black [5], cigarette smoke [6], and rural and urban air particulate [7,8] and generally co-occurs with B[a]P. CPP may be as much as 10-20 fold more abundant than B[a]P [9-11]. It is a potent bacterial mutagen and substantially more active than B[a]P [12]. Additionally, CPP is mutagenic in Chinese hamster ovary, V79 and human cells [13-15]. It is a strong inducer of adenocarcinoma in newborn mice [16], adenomas in weanling A/J mice [17], and is carcinogenic in mouse skin bioassays (Wood et al., 1980; Raveh et al., 1982). CPP is more potent than B[a]P as judged by mutagenicity to bacteria [12], malignancy of induced tumors [16], and tumorigenicity for A/J mouse lung [17].

Lee H. et al. (Chung Shan Medical and Dental College, personal communication) indicated that CPP contributes about 50% bacterial mutagenicity of total two-stroke motorcycle engine exhaust, which may be an important pollutant source in urban area of Taiwan due to the large amounts of two-stroke motorcycles display.

The principle DNA adducts have been characterized as *cis* forms of 3-(N2-guanly)-4-hydroxy-3,4-dihydroCPP diastereomers [18]. Major CPP metabolites, including 3,4-dihydroCPP-3,4-diol and 4-hydroxyl-3,4-dihydroCPP, can also be activated with sulfotransferase to react with DNA [19]. The availability of these standards aid identification of adduct(s) formed *in vivo* significantly. Generally, an immunochemical assay (ELISA), which is based on antibodies derived from adducts, provides more precise and feasible DNA-adduct quantification than the ^{32}P-postlabeling assay. Therefore, they are main focuses of this project. Certain factors have been shown to affect the formation of different diastereomeric DNA adducts and hydrolysis products of PAHs [20-22]. This hypothesis has been use to explain previous published data on CPP-DNA adducts [18], however, remains unproven. We were also able to test it in the current project.

三、研究報告(結果與討論)

This project establishes the HPLC

analysis of CPP-DNA adducts (Hsu et al., 1997). Certain modifications were made. First, adducts were generated in 10 mM sodium cacodylate buffer, pH 7.0, 5% THF (vol/vol), and 0.5M NaCl. Second, after H_2O and 10% MeOH elution for removing unreacted deoxyguanosine (up to 95%, estimated by HPLC) in Partisil 40 ODS-3 packed column, sole 70% MeOH was used to obtain the pyrene-containing fractions. Third, these fractions were first clarified by benzene followed by ethyl acetate extraction prior to HPLC analysis. Accordingly, the higher yields of *cis* CPP-deoxyguaonsinyl adducts were achieved (2.57% vs. 1.46% that was obtained from Hsu et al., 1997).

Two CPP-DNA adducts, at quantitative levels (approx. 1 mg each), have successfully been synthesized throughout the period of the project. They were characterized as the diastereomers of *cis*-3-(deoxyguaonsin-N2-yl)-4-hydroxy-3,4-dihydroCPP (Table 1). These synthetic standards are employing to obtain their corresponding antibodies to establish an immunochemical method, or ELISA, for detecting CPP-DNA adducts. ELISA is considered to be more suitable for adduct quantification relative to the ^{32}P-postlabeling technique.

We also investigated factors that may affect the formation of different stereoisomeric DNA adducts. More specifically, chloride ions, as the model compound for halide ions, were tested for diastereomeric CPP-DNA adducts. Chloride ion (0, 0.05, 0.1, 0.5M) was found to increase the proportion of *cis* adducts formed between CPPE and calf thymus DNA. The *cis* adduct/*trans* adduct ratio was elevated with the increasing concentrations of chloride in the reaction buffers. It is proposed that the increase in *cis*-adduct formation may be due to salt results from SN1 attack of chloide ion on the CPPE carbocation, forming a *trans* chlorohydrin, followed by SN2 attack of DNA.

四、計畫成果自評

We are interested in certain areas of research on environmental contaminant CPP, effects related to carcinogenesis in particular. In the current project, we established the HPLC analysis of deoxynucleoside conjugates and metabolites of CPP, acquired two major CPP-DNA adduct standards, and provided chemical basis for the preferential formation *cis*-CPP-DNA adducts.

In our ongoing NSC-supported project, we are in the process of making antibodies against the synthetic standards. In addition, we are examining distribution and clearance of various tissues, including blood, in CPP-treated animals. The result will aid us with determining if protein adduct detection deserves more attention as proteins are generally more abundant and easier access compared to DNA. To neglect the labile, i.e., unstable, DNA/protein adducts may lead to underestimate the carcinogenic risks of chemicals. Thus, they should not be overlooked. The likelihood of induction of cytotoxicity, e.g., apoptosis, and oxidative damage, e.g., 8-hydroxydeoxyguanosine, by CPP was also under investigation.

To the best of our knowledge, this is the first report attempting to gain understanding of the chemistry between CPP and DNA. Our findings suggest that chlorohydrin can be intermediates in the alkylation of nucleic acids by epoxide of CPP.

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Table 1. ^{1}H-NMR Data for CPPE-Deoxyguanosine Adducts[a]

	chemical shift	multiplicity
Gua1-NH	10.88	s
CPP H8[b]	8.36	dd
CPP H1	8.28	d
CPP H6[b]	8.27	dd
CPP H9	8.19	s
CPP H10	8.19	s
CPP H5	8.15	d
CPP H2	8.12	dd
CPP H7	8.08	dd
CPP H8	8.04	s
Gua 2-NH	7.11	d
CPP 4-OH[c]	6.32	d
Deoxyribose H1'	6.28	dd
CPP H3(CH-N)	6.14	dd
CPP H4(CH-O)[d]	5.84	dd
Deoxyribose 3'-OH[c]	5.20	d
Deoxyribose 5'-OH[c]	5.14	t
Deoxyribose H3'[e]	4.36	dddd
Deoxyribose H4'	3.79	ddd
Deoxyribose H5'[f]	3.55	ddd
Deoxyribose H5"[f]	3.49	ddd
Deoxyribose H2'	2.70	ddd
Deoxyribose H2"	2.29	ddd

[a] Spectra were recorded of solution in DMSO-D_6 on a Burker DRX-500 spectrometer at 27 ^{0}C. The values shown are for the adduct that eluted last from the reverse phase HPLC column.

[b] Assignments may be reversed.

[c] Not observed under conditions of fast proton exchange.

[d] Collapsed to a doublet under conditions of fast proton exchange.

[e] Collapsed to a doublet of doublets of doublets under conditions of fast proton exchange.

[f] Collapsed to a doublet of doublets under conditions of fast proton exchange.