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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 致癌機轉之研究
Carcinogenic Mechanisms of Cyclopenta[cd]pyrene

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主持人：陳叡瑜　　台北醫學大學公共衛生學系

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 進行體內（動物實驗）、外試驗，對於其可能之致癌機轉加以探討，主要研究方向包含瞭解 CPP 之去氧核醣核酸鍵結物 (DNA adduct) 及蛋白質鍵結物 （protein adduct）。成果包括 1) 瞭解 CPP 於動物組織分佈、排除，與血中主要蛋白質鍵結的情形，2) CPP 在動物體內與去氧核醣核酸、蛋白質結合能力的比較，以及 3) CPP 不穩定去氧核醣核酸鍵結物形成的情形。

關鍵詞：多環芳香碳烴、癌症、去氧核醣核酸鍵結物、蛋白質核酸鍵結物、不穩定鍵結物

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. CPP is also more potent than B[a]P as judged by mutagenicity and tumorigenicity.

The current project aims to investigate carcinogenic mechanisms of CPP. We examined DNA and protein adduct formation of CPP. CPP was found to bind to protein much stronger than to DNA in treated mice. We also analyzed the tissue distribution, clearance, and protein binding of CPP in a mouse model. Our findings also suggested that CPP formed unstable DNA adduct *in vitro*.

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

二、緣由與目的

Malignant neoplasms have been the first leading cause of death in Taiwan since 1982 and account for 24% of the total death in 1998. This figure has increased more than one-third since 1978, and is still increasing. Lung cancer is a leading cause of mortality in Taiwan (highest among women and the second among men) (Health Promotion and Protection, 1993). Cigarette smoking cannot explain the high lung cancer death in Taiwan women because less than 10% of this population is smokers. Therefore, environmental factors other than smoking may play an important role in lung cancer development, at least in female nonsmokers (Cheng et al., 2001).

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, i.e., genotoxicity, and exposure to specific carcinogens.

It is found that total DNA adducts in 86 Taiwanese lung cancer patients were much higher than those of other countries, and there was no difference observed on levels of DNA adduct between smoking and non-smoking lung cancer patients (Cheng et al., 1998). Moreover, 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. PAHs are major mutagenic and carcinogenic chemicals in airborne particulates. The amounts of PAHs, such as B[a]P and CPP, in airborne particulate samples of Taiwan are greater than those from other countries (Kuo el al., 1998). Taken together, understanding cancers, especially lung cancer, induced by PAH should be considered as an important environmental medical issue in Taiwan.

CPP, produced by incomplete combustion, is a widespread air contaminant. It is found in, for example, automobile exhaust (Stenberg et al., 1983), carbon black (Wallcave et al., 1975), cigarette smoke (Snook et al., 1977), and rural and urban air particulate (Grimmer et al., 1980; Nielsen, 1983) and generally co-occurs with B[a]P. CPP may be as much as 10-20 fold more abundant than B[a]P (Grimmer et al., 1977; Grimmer, 1979; Skopek et al., 1979). It is mutagenic in bacteria, Chinese hamster ovary, V79 and human cells (Eisenstadt and Gold, 1978, Wood et al., 1980; Raveh et al., 1982; Cavalieri et al., 1981). In addition, CPP is a strong inducer of adenocarcinoma in newborn mice (Busby et al., 1988), adenomas in weanling A/J mice (Nesnow et al., 1994), and is carcinogenic in mouse skin bioassays (Wood et al., 1980; Raveh et al., 1982). It is more potent than B[a]P as judged by mutagenicity to bacteria (Eisenstadt and Gold, 1978), malignancy of induced tumors (Busby et al., 1988), and tumorigenicity for A/J mouse lung (Nesnow et al., 1994). According to Dr. Lee H at Chung Shan Medical and Dental College, Taichung, Taiwan, CPP accounted for approx. 50% bacterial mutagenicity of total two-stroke motorcycle engine exhaust, which is an important pollutant source in urban area of Taiwan.

The principle CPP-DNA adducts have been characterized as 3-(N2-guanly)-4 -hydroxy-3,4-dihydro CPP diastereomers (Hsu et al., 1997). 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 (Hsu et al., 1999). We have successfully synthesized all the major CPP-DNA adducts in quantitative amounts in our previous NSC projects. These adduct standards will be used for identifying adducts formed *in vivo* and *in vitro* in the current report.

三、研究報告(結果與討論)

We investigated tissue disposition of CPP. The information helps us with determining what tissues to focus on DNA adduct analysis. We also determined the remaining CPP levels in various tissues when radioactive CPP was given to tested mice. Liver and skin retained the highest amount of radioactivity per unit amount of tissue, throughout the tested duration of 15 days. The clearance of the CPP from DNA in these tissues was also examined. Liver DNA exhibited the highest CPP binding throughout the tested duration of 15 days. In comparison, CPP bound to lung and skin DNA, depending on the day of analysis, with different preference.

Protein adduct to chemical exposures has been exploited as a surrogate marker of

DNA damage. There are several advantages to study protein adduct given that DNA is the target molecule for mutagenic events. First, proteins are usually present in greater abundance than DNA. If the adduct level of a protein is in the vicinity of that of DNA, there will be a much larger amount of material available for analysis. Second, proteins are not subject to enzymatic repair. Thus, the amount of adduct on a protein in general is a better representation of the dose than that on DNA when exposure to a carcinogen is chronic and intermittent. A supposed advantage of DNA is that it can represent the dose of the target organ. However, this advantage is lost when a surrogate cell is used, as is the case for leukocyte DNA from blood, mainly because repair capacity varies from one cell type to another.

If a protein forms stable adduct(s) and its turnover rate is predictable, there will be a defined mathematical relationship between dose and adduct level. Best-known examples are aromatic amines and hemoglobin (Hb), and alfatoxin B1 and serum albumin (Ab) (Skipper and Tannenbaum, 1990; Skipper et al., 1994). Other successful studies include that hemoglobin adducts used as dosimeters for detecting short-term or chronic exposure to fluoranthene (Gorelick et al., 1989). To date, most studied protein adducts are Hb and Ab adducts (Chang et al., 1994). The advantage of using Hb and Ab as biomarkers are that they meet criteria of ready availability, adduct stability, and defined turnover. To test if protein could serve as a biomarker and/or target for CPP exposure quantitatively, we measured CPP levels in blood proteins of treated mice. It is found that CPP bound to blood proteins 100 times more than to tissue DNA. Accordingly, more research on CPP-protein adducts seem to be warranted.

We found that the total adduct level increased linearly to the dose of CPP up to 500 ug/animal, but not in a higher dose level perhaps indicating a saturation of the activating system. The adduct level was determined by the radioactivity in the acid acetone precipitated globin collected five days after the dose.

Carcinogens can form unstable macromolecular adducts of carcinogens, and these labile conjugates may be as deleterious as stable adducts (Bailey et al., 1996a,b). Cancer risk of a carcinogen can be overly underestimated if levels of unstable adduct(s) were not taken into account. A number of carcinogens, including B[a]P and , form unstable DNA adducts (MacLeod et al., 1994; Barak et al., 1993 and references therein). Alfatoxin B1-N7-guaninyl adduct may be the best-studied case (reviewed by Essigmann et al., 1983). At neural and acid pH, the glycosidic bond of this adduct in DNA is easily hydrolyzed, resulting in an apurinic site. While at alkaline pH, the adduct rearrange through imidazole ring opening to give an alfatoxin B1-formamidopyrimidine derivatives, or FAPY. We are aiming at DNA and protein modification of CPP. The formation of unstable CPP adducts, hence, should not be neglected.

As a partial test of this inference, [^{3}H]CPP-3,4 epoxide (CPPE) was reaction with calf thymus DNA. Subsequently, extensive organic extraction and ethanol precipitation were adopted to ensure the purified DNA is free of CPP hydrolysis product(s) (Hsu et al., 1997). Modified DNA was then digested enigmatically or acid hydrolyzed into deoxynucleosides followed by analyzing using HPLC with radioactive detection. HPLC fractions of the enzymatic digest of [^{3}H]CPPE-adducted DNA were "stable" adducts, compared to our authentic standards. In a separated test, we applied neutral and/or acidic conditions, which are known to retain the integrity of nucleosides if not for labile adduct(s), to the adducted DNA. In this test, two fractions chromatographed differently from adducts from the enzymatic digestion. It is not irrational to speculate that they are apurinic products of unstable CPP-3,4 epoxide-DNA adducts.

As for protein conjugates, the disappearance of radioactivity measured in the total globin after the single dose of CPP to mice further supported the idea of unstable

adduct formation. A stable Hb adduct is expected to follow zero order kinetics in its clearance, decreasing in its concentration linearly with the life time of 40 days. However, the CPP adduct from thee Hb was cleared with a half-life of six days. This type of clearance pattern is not unusual. Hb adducts of several compounds including B[a]P have been shown to follow this pattern (Shugart, 1985).

四、計畫成果自評

We are interested in the carcinogenic mechanisms of CPP. Specifically, our focus was its DNA and protein adduct formation. We showed here that CPP bound to protein much stronger than to DNA. This result suggested that CPP-protein adducts deserve more attention as proteins are generally more abundant and easier access compared to DNA. Neglecting the labile, or unstable, DNA/protein adducts may lead to underestimate the carcinogenic risks of chemicals. In this study, we demonstrated that CPP could form unstable DNA adducts. We also present tissue distribution, clearance, and protein binding of CPP in a mouse model.

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