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貳、中文摘要

空氣懸浮粒子中含有很多致突變物或致癌物，其主要的來源大多是汽機車排放出來的廢氣，而主要成份分為兩大類:一為多環芳香烴(polycyclic aromatic hydrocarbons, PAHs)，二為硝基多環芳香烴(nitro-polycyclic aromatic hydrocarbons, nitro-PAHs)。而硝基多環芳香烴中又以 1-硝基多環芳香烴(1-Nitropyrene,1-NP)的含量最高，所以本實驗以氣管灌入法(intratracheal instillation)將化學藥物注射入 Sprague-Dawley rats (SD rats)的氣管，再輔以穿透式電子顯微鏡來觀察氣管上皮細胞的超微結構變化。

將 1-NP 與三氧化二鐵(Ferric oxide, Fe_2O_3)研磨後與二甲基氧化硫(dimethyl sulfoxide, DMSO)混合灌入 SD rats 的氣管，分成四組:第一組: 0.2 ml 0.9% 生理食鹽水、第二組: 3 mg Ferric oxide 與 0.2 ml DMSO、第三組: 3 mg 1-NP 與 3 mg Ferric oxide 與 0.2 ml DMSO、第四組: 5 mg 1-NP 與 5 mg Ferric oxide 與 0.2 ml DMSO，每週灌入一次，連續灌入十五週後犧牲動物取出氣管。結果顯示第一組呈現正常的氣管上皮細胞形態，第二組的杯狀細胞增加、細胞皺摺亦增加，第三組有化生(metaplasia)、細胞凋亡(Apoptosis)現象，第四組除了有化生、細胞凋亡外，還有基底膜不連續、不完整，有更惡化的現象，具有劑量依賴反應(dose-dependent response)。

總之，呼吸道是吸入致癌物的主要標的，而 1-NP **對** SD rats **產生的細胞病變是值得借鏡參考的，台灣近幾年來汽機車大量成長，相對所排放的廢氣中致癌物亦相對的增加，所以如何管制與預防應是降低肺癌或氣管疾病的主要辦法。**

參、英文摘要

The suspension particles in the air consist of numerous mutagens and/or carcinogens. One of the main sources of those harmful materials is mostly from the exhaust of motor-vehicles. They can be grouped into two main categories according to their chemical compositions, i.e. polycyclic aromatic hydrocarbons (PAHs) and nitro- polycyclic aromatic hydrocarbons (nitro-PAHs). Since the major component of nitro-PAHs is 1-NP, it was used as the main experimental testing reagent in this investigation. It is introduced into the trachea of Sprague-Dawley rats(SD rats) by intratracheal instillation. The inducible changes in tracheal epithelial ultrastructure were then monitored, using transmission electron microscopy.

The experimental animals were divided into 4 groups, suspensions of 1-NP at different concentrations was given to each group:(1) 0.2 ml 0.9% normal saline (NS)，(2) 3 mg Fe_2O_3 in 0.2 ml DMSO, (3) 3 mg 1-NP and 3 mg Fe_2O_3 in 0.2 ml DMSO, (4) 5 mg 1-NP and 5 mg Fe_2O_3 in 0.2 ml DMSO. Each group was introduced with the chemical once a week for 15 consecutive weeks. At the end of the 15th week , the animal were sacrificed and their tracheae were removed and processed for TEM observation.

Our results showed that animals in group 1 had normal morphology of tracheal epithelium. The number of goblet cells and the surface foldings in both lateral and basal surfaces significantly increased in group 2. Of the group 3, epithelial cells undergoing metaplasia and

apoptosis were observed. In group 4, not only the occurrence of cellular metaplasia and apoptosis were further increased, but the basement membrane also became discontinuous. The results seem to indicate a dose-dependent response might be involved.

During recent years, the number of motor-vehicles in Taiwan has been greatly increasing. The deterioration of air quality and the increase of carcinogens in the air become a great concern in our daily life. Since the respiratory tract is the main target of inspired carcinogens, the pathological alterations of tracheal epithelia caused by 1-NP can be used as a convincing yet perplexing evidence to show the importance of the relationship between the air quality and health. The control and prevention of the deterioration of air quality might be the answer of the increasing lung cancer and respiratory tract disorders.

肆、前言

近十幾年來台灣地區工商業發達、經濟成長迅速，各項建設突飛猛進，人口集中都會區，汽機車數量大幅成長，再加上郊區工廠林立，使得台灣地區空氣品質急速惡化。空氣污物來源相當複雜，包括工廠排放的廢煙、汽機車引擎排放的廢氣(Tuiominene et al.,1988)、露天燃燒廢棄物所產生的煙霧(Lee et al.,1994a)、甚至烹調時所產生的油煙(Qu et al.,1992)、拜神祭祖時所燃燒的拜香煙霧(Chen and Lee,1996)、或是抽煙時所產生的煙霧 (Lofroth et al.,1991)等都是造成空氣污染的重要污染源。根據衛生署統計資料(1998)顯示，自民國七十一年以來惡性腫瘤已成為台灣地區十大死亡原因之首位，其中又以肺癌死亡率增加的幅度最為顯著。因而研究肺癌發生率與空氣污染物的相關性，是近年來流行病學相當重要的研究課題之一，目前流行病學的研究已證實空氣污染和罹患肺癌是有相關性。例如在污染的環境中，肺癌的發生率確實有明顯增加的趨勢，當空氣污染物減少時，肺癌的發生率會降低(Rylander, 1990；Folinsbee, 1992；Lewtas, 1993)。所以推測空氣懸浮微粒中所發現的致癌物可能是造成肺癌的主要元凶 (Carnow and Meier, 1973 ; Menck et al., 1974)。

空氣污染物的來源相當複雜，但主要污染源是來自汽機車等引

擎所排放出來的廢氣，而工廠排放的廢氣以及人為燃燒垃圾廢棄物產生的懸浮微粒也是台灣地區空氣污染的重要來源 (Lee et al.,1994a) 。汽機車引擎排放的廢氣中有 30-40%是 1-硝基多環芳香烴(1-Nitropyrene, 1-NP)和二硝基多環芳香烴(dinitropyrene, DNP)(Tokiwa et al., 1986)，且在所有引擎廢氣中已有超過一百多種的含氮化合物(nitro compounds)被分析出來，而 1-NP 是含量最多的一種(Olah et al., 1981；Schuetzle 1983；Lewtas and Nishioka, 1990；Nishioka et al., 1982；Scheutzle et al., 1981)。

硝基多環芳香烴是一群廣泛存於環境中的污染物，為多環芳香烴(polycyclic aromatic hydrocarbons,PAHs)經硝化作用(Nitration)加上一個或多個硝基取代基而成的硝基多環芳香烴(nitro-polycyclic aromatic hydrocarbons,nitro-PAHs)。Fu (1990)等人指出在大氣環境中，硝基多環芳香烴可藉由下列兩種過程而生成： (1)在大氣中的自然反應下，多環芳香烴會與一氧化氮(NO)在大氣環境中，藉由光能量生成自由基，而進行硝化反應產生硝基多環芳香烴(Zielinska et al., 1990) 。(2)各種燃料燃燒不完全時所產生的二氧化氮離子(NO_2^+)離子會與多環芳香烴進行親電子性的硝化作用而生成硝基多環芳香烴。

目前已知四種硝基多環芳香烴 1-NP，1,3-二硝基多環芳香烴(1,3-DNP)，1,6-二硝基多環芳香烴(1,6-DNP)以及 1,8-二硝基多環芳香

烴(1,8-DNP)會在老鼠的乳腺、膀胱、腎臟、肝臟、肺臟等器官形成 DNA 鍵結物(DNA adducts) (Djuric et al., 1988；Norman et al., 1990)，且在動物實驗上也證實會誘發肉瘤、腫瘤、肺癌及鱗狀細胞癌等的形成 (Ohgaki et al., 1985；Tokiwa et al., 1986；Wislocki et al., 1986)。將 1-NP 以皮下注射注入於剛出生的雌性 CD 大白鼠的肩胛骨內，會在注射部位長出乳房腺癌(mammary adenocarcinoma)(Hirose et al., 1984, Imaida et al., 1991, Imaida et al., 1995)，另 1-NP 以口服方式給與剛斷奶的雌性 CD 大白鼠會長出乳房腫瘤(mammary tumor)(EI-Bayoumy et al., 1988, Imaida et al.,1991)，將 1-NP 由腹腔注入 CD 大白鼠，會產生肝腫瘤(McManus et al., 1984)。將 1-NP 加在培養的中國倉鼠卵巢細胞(Chinese hamster ovary cells, CHO cells)中，會造成姐妹染色分體交換率(sister chromatid exchanges,SCEs)增加(Nachtman and Wolff, 1982, Lafi and Parry, 1987)，加 1-NP 於老鼠氣管上皮細胞(rats tracheal epithelial cells, RTE cells)時，RTE 細胞的相對菌落形成效率(relative colony-forming efficiency, RCFE)(Mitchell and Thomassen, 1990)會降低，這些實驗均顯示出 1-NP 的毒性。另柴油車、汽機車、飛機等排放的廢氣 (Tokiwa et al., 1986; Robbat et al., 1986)，液化石油氣、氣體燃料的燃燒不完全 (Kinouchi et al., 1988; IARC 1898) 、影印機碳粉匣 (Lofroth et al., 1980)、甚至家庭用的瓦斯加熱器、煤炭的灰燼、

燒烤雞肉的熱油以及茶葉中 (Li et al., 1982; Dennis et al., 1984; Ohnishi et al., 1985)都可發現硝基多環芳香烴的存在。大部份的硝基多環芳香烴對細菌和哺乳動物細胞的致突變性可能是藉由硝基取代基的還原作用(nitroreduction)或環氧化作用(ring hydroxylation)後，形成的活化代謝物而鍵結上 DNA 所造成 (Chou et al., 1985；Delcolos et al.,1988; Mermelstein et al., 1981)。

所以本實驗以大白鼠(SD rats)為實驗動物，取空氣污染源中硝基多環芳香烴含量最多的 1-NP 以氣管灌入法(intratracheal instillation)(Saffiotti et al., 1968)灌入大白鼠氣管內，再輔以光學顯微鏡(Light microscope, LM)、穿透式電子顯微鏡(Transmission electron microscopy, TEM)觀察其氣管上皮細胞的結構變化。

伍、材料與方法

一、藥品與材料

品名	公司
Paraformaldehyde reinst for electron microscopy	Merck
Glutardialdehyde solution 25% for electron microscopy	Merck
Dimethylarsinic acid sodium salt trihydratse for electron microscopy	Merck
Sucrose (GR)	Sigma
Osmium tetroxide for electron microscopy	Merck
Ethanol absolute (GR)	Merck
1,2-Propylene oxide for electron microscopy	Merck
低黏度包埋劑 (Spurr' s resin)	Energy Beam Sciences
Toluidine blue (GR)	Sigma
Uranylacetat (GR)	Merck
Methanol (GR)	Merck
Lead(II) nitratse(GR)	Merck
Sodium citratse (GR)	Merck
1-Nitropyrene	TCI
Ferric oxide	Merk
Dimethyl sulfoxide	Merk
Sodium pentobarbital	ELC

包埋模板	TED PELLA
#200 **銅網**	Energy Beam Sciences
ILFORD **多重反差相紙**	ILFORD
玻璃條 25mm X 400mm	Agar Scientific Ltd.

二.方法

本實驗選用的動物為雌性 SD rats (Sprague-Dawley rats)約 200-250gm 共貳拾隻，將老鼠麻醉後以氣管灌入方式(intratracheal instillation)(Saffiotti et al., 1968)以餵食管緩慢的將化學藥物灌入氣管中，因 1-NP 的極性不高且在大氣中其固相會懸浮附著在懸浮微粒上，所以選用 DMSO 當做溶劑，而 Fe_2O_3 則當做 carrier。將實驗分成四組，第一組為對照組，只注射 0.9% 生理食鹽水 0.2 ml，第二組為 3 mg Fe_2O_3 研磨後與 0.2 ml 的 DMSO 混合，第三組為 3 mg Fe_2O_3 加 3 mg 1-NP 研磨後與 0.2 ml DMSO 混合, 第四組為 5 mg Fe_2O_3 加 5 mg 1-NP 研磨後與 0.2 ml DMSO 混合，每組五隻老鼠，每週灌入一次，連續灌入 15 週後分別犧牲取樣。以戊巴比妥鈉(sodium pentobarbital, 80 mg/kg)麻醉後，然後開胸腔，自左心室灌入 0.9%生理食鹽水，右心剪開將血引流出，直到血沖洗完全，再灌入固定液，灌流完全後，犧牲動物取出氣管，浸泡於固定液中。固定液為 4% paraformaldehyde、2.5% glutaraldehyde 溶於 0.2M 之 cacodylate 緩衝液中而成(pH7.3~7.4)。固定後以含 7% sucrose 之 0.2M cacodylate 緩衝液沖洗 30 分鐘，再以溶於相同緩衝液中之 1% 鋨酸(OsO_4)做後固定 (post-fixation)2 小時，再以緩衝液沖洗三次後，接著以漸進的酒精

50%、70%、80%、90%、95%二次、100%三次各 15 分鐘進行脫水，然後再以 propylene oxide 處理二次，每次 10 分鐘之後，即以包埋劑 Spurr' s resin 與 Propylene oxide 以 1：1 混合浸潤至第二天，再以純包埋劑進行包埋。

以上所有過程，均在室溫下進行。包埋完成後之組織塊，利用超薄切片機(Reichert Ultracut E)以玻璃刀切成厚片(0.5~1.0 μm)撈至玻片，以鑽石刀切成超薄切片(50~90 nm)後，撈至於銅網上，厚片以 Toluidine blue 染色，用光學顯微鏡(light microscope)觀察，超薄切片以醋酸鈾醯 (uranyl acetate) 及檸檬酸鉛 (lead citrate) 溶液作雙重染色後，以 JEOL JEM-1200EX 型穿透式電子顯微鏡(transmission electron microscope)進行觀察。

陸、結果

氣管是一個管狀構造，氣管壁上有一些不完整的軟骨環將氣管包圍住使氣管堅硬而不會塌陷，而氣管後壁的窄細條形區域沒有軟骨，此處軟骨兩端間的間隙由緻密的纖維膠原韌帶連結起來。氣管內面襯有纖毛柱狀上皮包括：1.纖毛細胞(ciliated cell)：呈柱狀，細胞質內含有粒線體、Golgi apparatus、lysosome、ribosome、ER 等，在細胞管腔表面有纖毛和微絨毛(microvilli)。2.基底細胞(basal cell)：為多角形小細胞位於基底膜上，細胞核占據胞體大部分，為一種不與管腔接觸的細胞，會形成幹細胞(stem cell)族群，可發展出其它細胞類型。3.中間細胞(intermediate cell)：可能是會轉形為纖毛細胞和杯狀細胞的幹細胞。4.杯狀細胞(goblet cell)：散布於纖毛細胞之間，能產生黏液，其分泌由局部性刺激控制包括溫度、濕度、吸入空氣中塵埃量、有害氣體等。5.神經內分泌細胞(neuroendocrine cell)：是一小的圓形細胞，核深染，細胞質澄清，位基底膜上。

Group 1：對照組 (Control)

SD rats 以 0.2% 0.2 ml 生理食鹽水(N.S)灌入後在光學顯微鏡和電顯下觀察支氣管上皮細胞呈圓柱狀(Fig. 1，2)，包含纖毛

細胞(ciliated cell)、杯狀細胞(goblet cell)和基底細胞(basal cell)等主要細胞，纖毛細胞占大多數，與相鄰的細胞間有接合複合體(junctional complex)連接(Fig. 3)，細胞間隙(intercellualr space)緊密，在管腔表面有微絨毛(microvilli)和纖毛(cilia)分佈，細胞質含有大量的粒線體(mitochondria)。基底細胞(basal cell)位於基底膜(basement membrane)上，為一種不與氣管管腔接觸的小型細胞，圓形的細胞核占細胞的大部份，細胞質含少量粒線體。杯狀細胞(goblet cell)則含有很多分泌顆粒(granules)，細胞質含高爾基體(Golgi apparatus)和粗糙內質網(rough endoplasmic reticulum,rER) 。

Group 2： Ferric oxide + DMSO-treated

SD rats 灌入 carrier-3 mg 三氧化二鐵(Ferric oxide, Fe_2O_3)顆粒經研磨後溶於 0.2 ml 二甲基氧化硫(dimethyl sulfoxide, DMSO)處理，在光學顯微鏡下觀察到杯狀細胞(goblet cell)增加(Fig. 4)，在電子顯微鏡下觀察到細胞間皺摺(folding)增加(Fig. 5)，細胞質中含有很多的粒線體(mitochodria)和MVB(multi-vesicle bodies)，在細胞基底面(basal layer)有指狀突起(cytoplasmic interdigitation)增加(Fig. 6)，基底膜(basement

membrane)呈現完整且連續。在杯狀細胞的細胞核呈分葉狀(segment)(Fig. 6，7)，分泌顆粒增加(Fig. 7)且纖毛細胞的粗糙內質網(rough endoplasmic reticulum,rER)有擴大(dilated)現象。所以加了 carrier-Fe_2O_3 後上皮細胞有些許的改變包括細胞間皺摺增加、basal layer 的 cytoplasmic interdigitation 增加和纖毛細胞的 rER 有擴大等現象。

Group 3：3 mg 1-NP + Ferric oxide + DMSO

SD rats 以 3 mg 1-硝基多環芳香烴(1-NP)與 3 mg Ferric oxide 研磨後溶於 0.2 ml DMSO 處理後，在光學顯微鏡下觀察到杯狀細胞增加(Fig. 8)且染色質有濃縮現象，在電子顯微鏡下觀察到纖毛細胞、杯狀細胞和基底細胞等細胞核的染色質(chromatin)濃縮聚集在核膜周圍，有明顯的核仁，細胞質指狀突起增加。杯狀細胞的顆粒突出於管腔中(Fig. 9)，部份細胞的細胞質呈現被溶解的現象稱此細胞為〝cellular ghosts〞(McCulloch et al., 1989)且細胞核分葉(Fig. 10，11)，部份細胞的細胞質呈捲曲(whorls) (Fig. 12)是細胞凋亡(apoptosis)的現象，在氣管上皮細胞表面有小泡(bleb)形成(Fig. 13，14)，直徑在 0.2-2 μm，且有明顯大的核仁且核仁散開亦是 apoptosis 的形

態。在細胞質內有明顯的纖維絲(filament) (Fig. 15)和胞橋小體(desmosome)增加(Fig. 16)表示細胞有扁平化的趨勢，當細胞凋亡體(apoptotic bodies)形成後會被鄰近的上皮細胞或吞噬細胞(macrophage)進行吞噬作用形成吞噬性細胞凋亡體(phagocytosed apoptotic body) (Fig. 17，18，19)，而吞噬性細胞凋亡體內包含了細胞核、纖維絲和完整的粒線體等胞器，與壞死(necrosis)後細胞內的胞器和細胞膜破裂是不同的，且細胞間隙增加和細胞指狀突起亦增加。細胞產生化生(metaplasia)現象，使原本為柱狀的上皮細胞形狀改變，且有明顯的核仁，細胞核呈分葉狀(Fig. 20)。所以灌入 3 mg 1-NP 後細胞產生化生現象外，杯狀細胞和 apoptosis 亦明顯增加，所以 1-NP 會對氣管上皮細胞造成傷害。

Group 4：5 mg 1-NP + Ferric oxide + DMSO

當 SD rats 以較高濃度 5 mg 1-NP 與 5 mg Ferric oxide 研磨後溶於 0.2 ml DMSO 處理後，在光學顯微鏡下可觀察到纖毛細胞減少、有 apoptotic body、細胞呈較扁平狀(Fig. 21)，在黏膜下層中有觀察到肥胖細胞(mast cell)(Fig. 22)明顯聚集。細胞呈現化生現象可觀察染色質濃縮分佈在核膜周圍，細胞核呈分葉

狀，且有細胞凋亡體(apoptotic body)(Fig. 23)即 rER 呈明顯捲曲(whorls)狀，且細胞間隙增加、染色質濃縮分散在細胞核周圍，細胞核分葉、且杯狀細胞的顆粒突出於管腔中(Fig. 24)，有明顯的纖維絲 (Fig. 25)分佈和 desmosome 增加(Fig. 26)表細胞有扁平化傾向。細胞質中游離核醣體(free ribosome)增加(Fig. 27)，基底膜(basement membrane)部份呈現不完整、不連續(Fig. 28)的現象，可觀察到上皮細胞的指狀突起伸展穿過基底膜至結締組織(Fig. 29)，亦有結締組織的細胞穿過基底膜至上皮細胞(Fig. 30)。且觀察到細胞核分裂增加、有 apoptotic bodies(Fig. 31)，細胞質剝離至管腔中(Fig. 32)且 rER 和高爾基體(Golgi apparatus)有明顯增加的現象(Fig.33)，表示有腺癌(adnocarcinoma)特徵的傾向。所以以較高濃度 5 mg 1-NP 灌入氣管上皮後除了有觀察到與 3 mg 1-NP 相同的結果外，另外還可觀察到基底膜斷裂不連續的現象，所以表示有更嚴重的傾向具有 dose-dependent response。

所以以 1-NP 灌入氣管後會造成上皮細胞喪失了原來柱狀上皮的形態即有化生(metaplasia)現象，且 apoptosis 明顯增加，另 free ribosome 、Golgi apparatus、 rER 明顯增加亦顯示蛋白質合成的需求在 apoptosis 是很重要而與 necrosis 有很大明顯的

差別。所以 apoptosis 時會有很 active 的 free ribosome 、Golgi apparatus、 rER 等來合成製造 membrane protein。

柒、討論

在正常大白鼠的支氣管上皮細胞的超微構造與狗(Frasca et al., 1968)、老鼠(Hansell et al.,1969)和人類(Rhodin,1966,Watson et al.,1964)是相同的，含有纖毛細胞(ciliated cell)、杯狀細胞(goblet cell)和基底細胞(basal cell)等細胞，ciliated cell 位管腔處有纖毛(cilia)、微絨毛(microvilli)，有一圓形細胞核，goblet cell 含有分泌顆粒，basal cell 含有一很大的細胞核占細胞的大部分，是不與管腔相鄰的細胞。當將 3mg 1-NP 和 5mg 1-NP 灌入大白鼠支氣管內時會造成細胞核與細胞質比例(N/C ratio)增加，細胞核變大、分葉甚至斷裂，核仁明顯、染色質(chromatin)濃縮聚集在核膜周圍，這一連串由 1-NP 造成細胞核明顯的傷害是癌化的一個重要細胞學診斷參考依據。

SD rats 以 3 mg 1-NP 和 5 mg 1-NP 處理後引起細胞間隙增加、細胞形狀改變、細胞核/細胞質比率(N/C ratio)增加、核仁變大且明顯、纖維絲增加等現象在人類的鱗狀細胞癌(squamous cell carcinoma)(Coalson et al., 1970; Greene et al., 1969)和因抽煙使得上皮細胞增生引起細胞膜剝落至管腔中(Gene et al.,1991;Frasca et al.,1968)等，都是使細胞走向病變和癌化前期的路徑。

Tarin (1969)認為當上皮細胞與結締組織間的接合改變在癌化的過程中是一個重要現象，所以當基底膜不連續、不完整時，且上皮細

胞的細胞突起(processes)侵入至結締組織時是癌症的前兆或是惡性腫瘤的徵兆(Tomakidi et al., 1999)，在乳房腺癌(Tarin ,1969)時亦會引起上皮細胞有相同的改變，在二氧化硫(Spiger et al., 1974; Lamb and Reid., 1968)的吸入，和抽煙(Lamb and Reid;1969)有相同的結果。

所謂化生(metaplasia)是指由一細胞形態轉變成另一細胞形態的過程，在本實驗中的第三組和第四組都有觀察到此現象，且 goblet cell 有增加的趨勢，胞橋小體(desmosome)和纖維絲(filament)都有增加，表示細胞有扁平化的趨勢即有表皮樣化生(epidermoid metaplasia) 的傾向(Harris et al., 1976)，但因為有分化良好的內質網和高爾基體所以屬於腺癌(adenocarcinoma)的分類，依據 WHO(Kreyberg, 1967)對肺癌的六大分類中應屬於第五類：即合併表皮樣和腺癌(Combined epidermoid and adenocarcinomas)，因有明顯的纖維絲(filament)與胞橋小體(desomosome)歸類於扁平樣(epidermoid)的化生，而有分化良好的高爾基體(Golgi apparatus)和粗糙內質網(rER)則歸類於腺癌(adenocarcinoma)。

1-NP 經 microsome 和 cytosol 的 nitroreductase、ring hydroxylation、acyltransferase 等作用後，所產生的代謝產物有很多種，而最後代謝產物的活性、毒性等因標的細胞(target cell)(Kitamura and Tatsumi,1996; Howard et al., 1995; Heflich et al., 1990; Lafi and Parry, 1987)不同而有差異，如 CHO cell 以 1-NP 處理後，其代謝物中

6-hydroxyl-1-nitropyrene, 1-nitropyrene 9,10-oxide 和 1-nitropyrene 4,5-oxide 等具有很強的細胞毒性，當金魚以 1-NP 處理後，其代謝物中以 1-aminopyrene 的含量最高，所以被活化的代謝物與 DNA 結合而產生 DNA 鍵結物(adducts)所造成傷害程度亦會有所不同，所以以同樣藥物處理不同的實驗動物時會有不同的反應。

當 1-NP 注入 SD rats 氣管後，染色質濃縮且聚集在核膜周圍似鐘面(〝clock-face〞)、細胞核的形狀不一致、細胞壓縮使得細胞間隙加大、管腔內的纖毛消失、內質網擴張、在管腔有細胞小泡(bleb)、核仁分裂、纖維絲增加，最後膜性的細胞凋亡體(membrane-bound apoptotic bodies)，最後細胞凋亡體(apoptotic bodies)會被結締組織中的巨噬細胞(macrophage)或鄰近的上皮細胞吞噬(phagocytosis)後形成吞噬性細胞凋亡體(phagocytosed apoptotic bodies)，其內含有細胞質、細胞核殘餘和部份胞器，phagocytosed apoptotic bodies 會被排出至管腔等細胞凋亡(apoptosis)現象(Clarke and Clarke,1995; Cummings et al., 1997; Kwong et al.,1999; Kaneko et al.,1999)。apoptosis 在生理上和發育過程有著相當重要的地位且在不同的病理疾病中亦扮演了重要的參與角色(Walker et al., 1988; Wyllie et al., 1980)，除了形態學上觀察到的變化外，在生化上亦可觀察到因去氧核醣核酸(DNA)斷裂成約 200bp 之整數倍的片段，在電泳(agarose gel electrophoresis)時會產生

階梯(ladder pattern)的結果(Whllie, 1980)，且 p53 和 bcl-2 兩基因亦參與 apoptosis，另肥胖細胞(mast cell)產生眾多調節因子(mediators)中的 TNF(tumor necrosis factor)(Plaut et al., 1989; Angermuller et al.,1998) 亦會使細胞產生 apoptosis(Shen and Shenk, 1995)。

本實驗因時間和技術上的限制，若能再配合其他分生技術將 apoptosis 加以量化和使用抗原、抗體特異性標示的功能，將能解決更多的問題。

總而言之，1-NP 對 SD rats 產生的細胞病變有 metaplasia、apoptosis、甚至基底膜不連續等更具侵襲性的病變結果，是值得重視的。在現今台灣工廠林立、汽機車大量成長的同時，相對的排放廢氣中致癌物亦相對增加，所以如何管制和預防應是降低逐年增加肺癌或氣管疾病的主要辦法。

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玖、圖表與說明