UNIVERSITI PUTRA MALAYSIA

STUDIES ON THE EFFECTS OF BENZ [a] ANTHRACENE AND BENZO [ghi] PERYLENE ON THE LUNG OF RATS

SANAZ MOVASSAGH

FPV 2003 6

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By

SANAZ MOVASSAGH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

January 2003

SAY, O' MY LORD! ADVANCE ME IN KNOWLEDGE
Holy Quran (Surah Taha-144)

To my beloved spouse for all what he do and done for me

Thankfulness and Gratefulness

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Master of Science.

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Chairman: Associate Professor Noordin Mohamed Mustapha, Ph.D.

Faculty: Veterinary Medicine

Air pollution or haze contains a variety of detrimental agents including benz[a]anthracene (BA) and benzo[ghi]perylene (B[ghi]P). These carcinogenic compounds which are found during the Malaysian haze episodes affected on health and economic status of nation. In view of developing strategies and bringing about a remission of noxious haze effects in humans, acute and chronic exposure to BA, B[ghi]P and their combination was studied in rats.

The acute exposure studies was conducted to evaluate apoptosis in the lung of rats following treatment with BA, B[ghi]P and BA+B[ghi]P. Rats not receiving any treatment served as control while those administered with BA, B[ghi]P and BA+B[ghi]P were instilled intratracheally at the dose of 8 ng (4 µl), 16 ng (8 µl) and 12 ng (6 µl) respectively. Animals in treated groups were euthanised at 1, 4, 8, 16 and 24 hours post-instillation (p.i.) and rats from the control group were only killed 24 hours p.i. Apoptosis assessment was made on haematoxylin-eosin stained histologic sections, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) analysis and DNA laddering of trachea and lung samples.

Different stages of apoptosis were found in the pneumocyte and bronchial epithelium of all treated rats euthanised at the respective time post-instillation and the percentage of apoptotic cells increased with advancement of time, especially in 16 and 24 hours p.i. which was the highest for each group ($P<0.05$). This was also confirmed by TUNEL analysis and DNA laddering.

The chronic exposure studies was conducted on changes in the lung of BA, B[ghi]P and BA+B[ghi]P-induced rats during a three month period encompassing gross and histopathologic findings, immune response, levels of marker enzyme and the effect of raw garlic as an anti-tumour factor.

Rats from the control, BA, B[ghi]P and BA+B[ghi]P group were daily fed on a commercial basal diet while rats from the garlic (G), [BA+G], [B[ghi]P+G] and [BA+B[ghi]P+G] group were fed the basal ration containing garlic incorporated at the rate equivalent to an intake of 80 mg/kg body-weight/rat/day.

The results showed growth disturbances in pneumocytes and bronchial epithelium of rats from the BA, B[ghi]P and BA+B[ghi]P groups. Apoptosis was also detected in these groups. In addition, rats treated with BA+B[ghi]P had the lowest levels of IgA, IgG, alveolar macrophages activities and glutathione S-transferase in the lung.

In conclusion, environmental hazard of BA, B[ghi]P and their combination to lungs of rats as target organs causes deleterious changes either by short or long term exposures and garlic has a great potential in alleviating the chronic effects.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

KAJIAN MENGENAI KESAN BENZ[a]ANTHRACENE DAN BENZO[ghi]PERYLENE PADA PARU-PARU TIKUS

Oleh

SANAZ MOVASSAGH

Januari 2003

Pengerusi: Profesor Madya Noordin Mohamed Mustapha, Ph.D.

Fakulti: Perubatan Veterinar

Pencemaran udara atau jerebu mengandungi pelbagai jenis agen yang merbahaya termasuk benz[a]anthracene (BA) dan benzo[ghi]perylene (B[ghi]P). Semasa Malaysia dilanda masalah jerebu, bahan-bahan karsinogen ini didapati membawa kesan kepada kesihatan dan status ekonomi negara. Kajian mengenai pendedahan akut dan kronik kepada BA, B[ghi]P dan gabungannya telah dijalankan ke atas tikus bagi merancang strategi mengurangkan kesan merbahaya terhadap manusia.

Dalam kajian pendedahan akut kepada rawatan BA, B[ghi]P dan BA+B[ghi]P, penilaian terhadap apoptosis pada paru-paru tikus telah dibuat. Tikus yang tidak menerima sebarang rawatan bertindak sebagai kawalan, manakala yang dirawat menerima dos 8 ng (4µl), 16 ng (8 µl) and 12 ng (6 µl) yang masing-masing diberi secara intratrakea. Tikus dari kumpulan menerima rawatan dibunuh pada 1, 4, 8, 16 dan 24 jam pasca-pemberian (p.i). Penilaian apoptosis dijalankan secara histologi dengan haematoxylin-eosin, analisis pelabelan potongan hujung TUNEL dan tangga DNA pada sampel trakea dan paru-paru.

Apoptosis pada pelbagai peringkat telah dijumpai pada pneumosit dan epitelium bronkiol tikus yang dibunuh pada p.i. yang berasingan, di mana peratusan sel apoptosis meningkat sekadar dengan masa, terutamanya pada 16 dan 24 jam p.i. yang mencatat paling tinggi dalam setiap kumpulan ($P<0.05$). Ini turut dikenalpasti positif dengan TUNEL dan tangga DNA.

Pada kajian pendedahan kronik, perubahan pada paru-paru tikus yang dirawat dengan BA, B[ghi]P dan BA+B[ghi]P selama 3 bulan menunjukkan perubahan histologi yang jelas, gerakbalas imun, aras enzim petunjuk dan kesan bawang putih mentah sebagai faktor anti-tumor.

Tikus dari kumpulan kawalan, BA, B[ghi]P dan BA+B[ghi]P diberi makanan komersial manakala tikus dari kumpulan bawang putih (G), [BA], [B[ghi]P] dan [BA+B[ghi]P] diberi makanan yang telah ditambah dengan bawang putih mentah pada kadar 80 mg/kg berat badan/tikus/hari.

Keputusan kajian menunjukkan gangguan tumbesaran pada pneumosit dan epitelium bronkiol pada tikus BA, B[ghi]P dan BA+B[ghi]P. Apoptosis turut dikesan pada kumpulan ini. Tambahan pula, tikus yang dirawat dengan BA+B[ghi]P menunjukkan aras IgA, IgG, aktiviti makrofaj alveolus dan gluthathione S-transferase yang rendah pada paru-paru.

Kesimpulannya, pencemaran yang merbahaya dari BA, B[ghi]P dan gabungannya terhadap paru-paru tikus sebagai organ sasar boleh menyebabkan

perubahan, mahupun pada jangka masa pendek atau panjang dan bawang putih mentah mempunyai potensi yang besar untuk mengurangkan kesan kronik BA dan B[ghi]P.

ACKNOWLEDGEMENTS

I am grateful to be given an opportunity to express my profound thanks and appreciation to Associate Professor Dr. Noordin Mohamed Mustapha, the chairman of my supervisory committee for his supervision and continuing guidance, support and enthusiasm, advice and constructive criticism by giving greater latitude of freedom in conducting this study as well as completion of this thesis. I never can repay him back all his favour.

I would also like to extend my gratitude and thanks to Professor Dato' Sheikh Omar Abdul Rahman, a member of my supervisory committee for his concern, encouragement and his valuable time to guide me during my work.

I would like to express my thanks to the other member of my supervisory committee, Dr. Hassan Hj. Mohd Daud for his comments, help and allowing me the use of his laboratory on my molecular work.

My deeply appreciation and special thanks to Dr. Abdul Rahman Omar, who provided facilities in his laboratory during of this project. Thanks for his kind assistance and invaluable technical guidance.

My sincere thanks are due to University Putra Malaysia (UPM) for the financial support through IRPA fund for this research. A special acknowledgement and sincere thanks to all the staff in the Faculty of Veterinary Medicine for their cooperation.

I am also indebted to Mr. Kufli Che Noor and to Mr. Kumar Rajabopal for managing animals and all staff of the Post Mortem laboratory. My special appreciation is also extended to my colleagues especially to the Biology lab members, Post Mortem lab and in Aquatic Animal Health Unit. Thanks and appreciation also goes to my dear friends especially Miss Abeer Sahtout, Miss Tay Bee Hong, Dr. Muthafar Al-Haddawi, Dr. Thoria, Dr. Karim Alwan, Mr. Albert Gabrael and all of my country-mate's friends. My thanks to Mr. Wan Keng Fei for the Bahasa Malaysia translation of the abstract.

Last but not least, I wish to express my appreciation to my wonderful parents who are always beside me for their prayers and patience, unconditional love, inspiration and support whenever I need, to my sister Hedieh for her attention and moral support and grateful thanks to my husband Hamid without him the completion of this degree would have been impossible.

Finally my thanks to any one who has helped in one way or another toward this degree. Thank Allah for giving me the patience and guidance with wisdom and strength for completing my study.

I certify that an Examination Committee met on 31st January 2003 to conduct the final examination of Sanaz Movassagh on her Master of Science thesis entitled "Studies on the Effects of Benz[a]Anthracene and Benzo[ghi]Perylene on the Lung of Rats" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

JASNI SABRI, Ph.D.
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

NOORDIN MOHAMED MUSTAPHA, Ph.D.
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

DATO' SHEIKH OMAR ABDUL RAHMAN, M.R.C.V.S.
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

HASSAN HJ. MOHD DAUD, Ph.D.
Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

SHAMSHER MOHAMAD RAMADILI, Ph.D.
Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 6 FEB 2003

The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment for the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

NOORDIN MOHAMED MUSTAPHA, Ph.D.
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

DATO' SHEIKH OMAR ABDUL RAHMAN, M.R.C.V.S.
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

HASSAN HJ. MOHD DAUD, Ph.D.
Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

AINI IDERIS, Ph.D.
Professor/ Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 13 MAR 2003

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

SANAZ MOVASSAGH

Date: 6.2.2003

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LIST OF ABBREVIATIONS

AGE	Aged garlic extract
AHH	Arly hydrocarbon hydroxylase
Ahr	Aryl hydrocarbon receptor
AMØ	Alveolar macrophage
ANOVA	Analysis of variance
BA	Benz[a]anthracene
BALT	Bronchial associated lymphoid tissue
B[ghi]P	Benzo[g,h,i]perylene
CV	Crystal violet
CYPs	Cytochrome P-450 isoforms
CYP1A1	Cytochrome P-450 1A1
CYP2E1	Cytochrome P-450 2E1
DAS	Diallyl sulfide
DASO	Diallyl sulfoxide
DASO(2)	Diallyl sulfone
DADS	Diallyl disulfide
DMBA	7,12-dimethylbenz(a)anthracene
DNMRT	Duncan's New Multiple Range Test
G	Garlic
GGMSC	Gamma-glutamyl-Se-methylselenocysteine
H&E	Haematoxylin-eosin
hr	Hour
LC	Langerhans'cells
MBAs	Monomethylbenz(a)anthracenes
MDR	Multidrug resistance
MSC	Methylselenocysteine
OD	Optic density
PAHs	Polycyclic aromatic hydrocarbons
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PDT	Photodynamic therapy
p.i.	Post instillation
PM	Particulate matter
RBC	Red blood cell
SAC	S-allyl cysteine
SE	Standard Error
TdT	Terminal deoxynucleotidyl transferase
Tr.	Tricaprylin
TUNEL	Terminal deoxynucleotidyl transferase- mediated dUTP nick-end labeling

CHAPTER I

INTRODUCTION

Air pollution, which is not a recent phenomenon, provides a wide variety of injurious contaminant chemicals for humans, animals and plants. Suspended particles dispersed through a portion of the atmosphere can cause haze. Combustion products, organic compounds, metals, acid aerosols and cigarette smoke as airborne particulate can interfere with the normal defense processes and metabolic systems of the human body to enhance the incidence of cancer (Gilmour *et al.*, 2001). Among the harmful chemicals released to environment is a class of very stable organic molecules made up of only carbon and hydrogen known as polycyclic aromatic hydrocarbons (PAHs), commonly found in the emissions from domestic, agricultural and industrial settings (Vaessen *et al.*, 1988; Holman, 1999). Exposure to different levels of PAHs is connected with deleterious short and long-term effects on human health (Kramers and Van Der Hijden, 1990). One of the most carcinogenic PAHs is benz[a]anthracene (BA), which is a major component of environmental pollutant present in smoke and flue gases, coal tar, petroleum and organic material burning (Sittig, 1985). Benzo[g,h,i]perylene (B[ghi]P) is also among the carcinogenic PAHs found during natural fires and present in edible oils, cigarette smoke and coal tar (IARC, 1983).

It has been reported that a significant increase in particulate matter concentration produced in polluted regions, can lead to variation in metabolism genes (Lan *et al.*, 2000). High levels of (PAH)-DNA adduct is an indicator for occurrence of lung cancer associated with the rate of mortality caused by indoor coal-burning (Xu *et al.*, 1997).

Exposure studies to BA in animals have yielded skin cancer (Rice *et al.*, 1988) besides embryotoxicity (Christou *et al.*, 1990). Also studies in animals have shown B[ghi]P simultaneously increases incidence of skin tumours when administered with Benzo[a]pyrene (Van Duuren *et al.*, 1973). Effects from BA on lung have shown other kinds of tumours (Klein, 1963), as well as from B[ghi]P (Deutsch-Wenzel *et al.*, 1983). Furthermore, it have proved that B[ghi]P and BA to be mutagenic (Hughes and Phillips, 1993; Whong *et al.*, 1994).

Although data relating chronic or sub-chronic toxicity resulting from exposure of humans to BA is identified, no relevant reports of human effects exposure to B[ghi]P is known. Likewise, direct evidence of acute toxicity for humans in these cases is not available (RAIS, 1997a; 1997b). Some PAHs such as BA are also immuno-suppressors (Saas *et al.*, 1996).

The effect of air pollution on health is a developing issue in the whole world (Anderson, 1999). There are many epidemiological and experimental studies about the health effects of air pollution (Ostro *et al.*, 1999; Rosales-Castillo *et al.*, 2001; Hwang and Chan, 2002).

Regarding to the haze episodes in Malaysia, especially dense one occurred in 1997, there has been considerable interest about pollutant effects arising from it. However, the effect of most of air pollutants on lung function in human or animals is lack of study despite their carcinogenic potentials.

The aim of this study was to determine the effect of haze in humans using rats as models. It is also aimed at evaluating environmental pollution caused by haze and the relationship between the BA and B[ghi]P on the respiratory system.

It is hypothesized that BA and B[ghi]P exert deleterious effects on the respiratory system and this can be alleviated by oral supplementation of garlic. Hence, this study is conducted to assess the effect of BA and B[ghi]P administration on rats based on the following objectives:

1. to determine the manner of cell death during acute exposure to BA and B[ghi]P in lung tissue
2. to detect the presence of lung tumours during chronic exposure
3. to determine the sensitive and reliable indicators of tumours induced by BA and B[ghi]P
4. to assess the efficacy of garlic in alleviating BA and B[ghi]P induced injury

CHAPTER II

REVIEW OF LITERATURE

Air Pollution

The impact of air pollution to human health and economic status of nation is one of the important issues all over the world. Air pollution can be defined as any atmospheric condition that consists of a mixture of numerous compounds emitted by many distributed sources and present above normal ambient levels to produce measurable effects on man, animal, vegetation or materials. The impacts of air pollution encompass animal, public and ecosystem health. Air pollution, both indoors and outdoors, is a significant cause of health problems (Painter, 1974).

The pollution source can be referred to biological, chemical or economical by-products, natural or anthropogenic, vary between biomass burning, volcanoes, wildfires, fuel combustion, smoke, dust and radioactive gases (Elsom, 1992).

Air pollution can be transported hundreds of miles downwind from its origin. Since it cannot recognize political boundaries, states and communities cannot independently solve the pollution problems (OAQPS, 2000).

Haze is a phenomenon defined as tiny particulate suspended in air, originating from large-scale forest and land fires, which reduces visibility due to the scattering of light caused by aerosols. Based upon the experiences, the highest levels of haze are in

winter and lowest in summer (Heil, 2000). It is caused in large part by man-made air pollutants (ARB's, 2000).

Particulate matter (PM) consist of carbon and mineral bodies of different sizes, produced by the burning of wood, fuels and industrial process, floating in the air and exist as gases, liquid drops or solid particles. They are small enough to be inhaled into the deepest parts of the lung and often grow in size as humidity increases (Heil, 2000).

Ambient air particulate matters are classified into two distinct modes in distribution, namely the coarse and fine particles (Hsiao *et al.*, 2000). While breathing, particles are retained according to their size within the respiratory system, from upper respiratory tract to deeper, which impact on the human health especially elderly people and young children. The smaller are the particles, the more surface they offer for other pollutants to adsorb on explaining why smaller particles generally contain a higher amount of harmful compounds (Heil, 2000).

The PM_{10} is a particle with diameter less than or equal to 10μm and $PM_{2.5}$ has diameter less than 2.5μm. These particles are primary particulate of air pollution that threatens both health and environment (Heil, 1998).

Epidemiological studies have demonstrated an association between different levels of air pollution and various health outcomes including mortality, exacerbation of asthma, chronic bronchitis, respiratory tract infections, ischaemic heart disease and stroke. Air pollution increases the risk of chronic diseases (Kunzli *et al.*, 2001). Acute

effects of air pollution exposure include irritation of the nose and eyes, lung function changes, respiratory changes, headache, fatigue and nausea and chronic exposures are associated with cough, sputum production and lung function decrements (Sydbom *et al*., 2001).

Polycyclic aromatic hydrocarbons (PAHs) are a group of large organic molecules found as mixtures formed as a result of incomplete combustion. The physical and chemical properties of PAHs are not all alike, nor are their health effects. A common mechanism of transport is by spontaneous desorption and transfer through the aqueous phase. Plant *et al*. (1983) showed that molecular volume of PAHs is a rate-determining factor for transfer. The PAHs interact with the aryl hydrocarbon receptor (Ahr) to cause reproductive defects (Matzuk, 2001).

Many PAHs have been identified as cancer-inducing chemicals in animals or humans (Karahalil *et al*., 1999). One pollutant with low carcinogenic effect can increase the effect of another one (Van Duuren *et al*., 1973; Van Duuren and Goldschmidt, 1976).

The primary source of emitted PAHs is incomplete combustion of wood or fuel. Sources include motor vehicles, non-road vehicles and equipment, residential wood combustion, industrial and commercial combustion, coke ovens, cigarette smoke, primary aluminum production, petroleum catalytic cracking, and charcoal broiled foods. The PAHs accumulate in terrestrial and aquatic life but can be metabolised by many plants and animals (EPA, 2000). Effects of PAHs reported in animal studies include