UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF BENZO (A) PYRENE (BAP) ON THE RESPIRATORY TRACT OF DOGS

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Thesis Submitted in Fulfilment of the Requirement for the
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September 2000

DEDICATION

This thesis is dedicated with appreciation to my

Husband, father and mother, father and mother- in- law, Wan,

Abang, Along, Mie, Kak Yan, Dr. Lan, Dr. Rina, Imah, Ayoi, Ijam and Adik Wan,

who provide my inspiration,

and also not forget to

Nabilah Huda, Nurul Farahana Hazira, Nurul Hanis Fazliana and Mohd. Afiq:

"May the understanding of these impacts reduce the

burden they impose on all our lives".

-WATI-

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

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Chairman: **Dr. Noordin Mohamed Mustapha**

Faculty: **Veterinary Medicine**

The global impact of air pollution encompasses the population health and the economic status of a nation. Air pollution or 'haze' contains a variety of noxious agents including benzo(a)pyrene (BaP). This compound is known to induce acute or chronic deleterious effects. The objectives of the study were to determine the effect of BaP on the physiology, defense mechanism and pathology of the lung, to suggest sensitive diagnostic techniques for the diagnosis of early carcinogenesis and to recommend preventive measures to minimise the effect of BaP.

An experiment was conducted in 27 dogs that are allocated to nine groups simulating different environmental condition and health status. The groups comprising of three dogs each were as follows: control, BaP, cyclosporine (Cyclo), Selenium (Se), BaP+Cyclo, BaP+Se, BaP+Cyclo+Se and Tricaprylin (Tri). Benzo(a)pyrene was given at the dose of 120 ηg/dog intratracheally twice, six week apart, Se 20 μg/dog/day and cyclosporine at the dose 50 mg/m^2. The tidal volume (Vt) and whole blood glutathione peroxidase (GSH-PX) activity was analysed weekly for 12 weeks. While at necropsy, bronchoalveolar lavage (BAL) cytology, alveolar macrophage (AMØ) activities, BAL immunoglobulin (Ig) G and Ig A level, gross and histopathology of lungs were also analysed.

The finding revealed that the tidal volume (Vt) remain unchanged in all groups during the experimental period. The pulmonary immune response includes AMØ number, phagocytic and intracellular killing activities, and Ig A level in bronchoial alveolar lavage (BAL) that was markedly suppressed in the BaP, Cyclo and BaP+Cyclo groups. Subsequently, the BaP and BaP+Cyclo+Se group exhibited gross and microscopic appearance of tumorigenesis, which was diagnosed as pulmonary adenocarcinoma with expression of mutant p53 protein while the BaP+Cyclo and BaP+Se had atypical adenomatous hyperplasia (AAH).

Based on the finding, exposure to BaP can lead to pulmonary immuno-suppression and tumorigenesis in dogs. It is also showed that during haze episode, immuno-stressed

individuals are more prone to the development of pulmonary immuno-suppression and tumorigenesis. Selenium supplementation or cyclosporine has great potential in combating these deleterious effects. However, simultaneous supplementation of Se together with cyclosporine during haze is not advised, since this will promote tumorigenesis in the lung.

In conclusion, intratracheal instillation (twice, six week apart) of 120 ηg BaP/dog causes insignificant reduction of Vt, pulmonary immunosuppression and pulmonary carcinogenesis. The immunocytochemical detection of p53 can be used as a sensitive diagnostic technique for the diagnosis of early pulmonary carcinogenesis. Daily oral administration of Se as a supplement has great potential in minimising the adverse effect of BaP.

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

KESAN BENZO(A)PYRENE KE ATAS SISTEM PERNAFASAN ANJING

Oleh

HAZILAWATI HAMZAH, D. V. M.

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Kesan global pencemaran udara memudaratkan kesihatan populasi dan juga status ekonomi negara. Pencemaran udara atau 'jerebu' mengandungi berbagai jenis bahan-bahan merbahaya termasuk benzo(a)pyrene (BaP). Bahan ini diketahui boleh menyebabkan kesan akut dan kronik yang merbahaya. Tujuan ujikaji ini dijalankan adalah untuk menentukan kesan BaP ke atas fisiologi, mekanisma pertahanan dan patologi paru-paru, untuk mencadangkan teknik diagnosis yang sensitif untuk diagnosis awal barah paru-paru dan mencadangkan untk mencadangkan langkah-langkah pencegahan bagi meminimumkan kesan BaP.

Ujikaji telah dijalankan pada 27 ekor anjing yang telah dibahagikan kepada sembilan kumpulan berdasarkan ke atas simulasi persekitaran dan status kesihatan yang berbeza. Kumpulan-kumpulan tersebut yang masing-masing mempunyai tiga ekor anjing adalah: kontrol, BaP, siklosporin (Cyclo), Selenium (Se), BaP+Cyclo, BaP+Se, BaP+Cyclo+Se dan Trikaprilin (Tri). Benzo(a)pyrene (BaP) telah disuntikkan pada dos 120 ηg/anjing secara intratrakea sebanyak dua kali berselang enam minggu. Isipadu tidal dan aktiviti glutation peroksidase (GSH-Px) darah telah dianalisis setiap minggu selama 12 minggu. Sitologi dan basuhan bronkiol alveolus (BAL), aktiviti makrofaj alveolus (AMØ), tahap immunoglobulin (Ig) G dan Ig A dalam BAL, patologi makro dan mikro paru-paru telah dianalisis semasa nekropsi.

Hasil kajian menunjukkan isipadu tidal (Vt) kekal tidak berubah di dalam semua kumpulan sepanjang jangkamasa ujikaji. Tindakbalas keimunan paru-paru termasuk jumlah AMØ, aktiviti fagositosis dan pembunuhan intrasel, dan paras Ig A di dalam BAL menunjukkan perubahan yang sangat ketara dalam kumpulan BaP, Cyclo dan BaP+Cyclo. Seterusnya, kumpulan BaP dan BaP+Cyclo+Se menunjukkan pembentukkan barah paru-paru secara kasar dan mikroskopi, yang mana telah didiagnosis sebagai adenokarsinoma pulmonari dengan kemunculan protin mutan p53, sementara itu kumpulan BaP+Cyclo dan BaP+Se mempunyai hiperplasia atipikal seperti adenoma (AAH).

Berdasarkan kepada penemuan ini pendedahan kepada BaP boleh menyebabkan penurunan keimunan pulmonari dan pembentukkan barah pada anjing. Ini juga

menunjukkan bahawa semasa jerebu, individu yang mempunyai tahap keimunan yang rendah lebih mudah terdedah kepada menurunan keimunan pulmonari dan pembentukkan barah. Pengambilan Se atau siklosporin mempunayi potensi yang besar untuk melawan kesan bahaya ini. Walau bagaimanapun, pengambilan Se serentak bersama siklosporin semasa jerebu adalah sangat tidak digalakkan kerana ia akan merangsangkan pembentukkan barah di dalam paru-paru.

Kesimpulannya, suntikan BaP secara intratrakea (dua kali, berselang enam minggu) pada dos 120 ηg/anjing menyebabkan penurunan Vt yang tidak ketara, penurunan mekanisme pertahanan paru-paru dan pembentukan barah pulmonari. Pengesanan p53 secara immunositokimia boleh digunakan sebagai satu teknik diagnosis yang peka bagi karsinogenesis awal. Pengambilan tambahan Se secara oral setiap hari mempunyai potensi yang besar untuk meminimumkan kesan buruk BaP.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS

AAH	atypical adenomatous hyperplasia
ADCC	antibody-dependent cellular cytotoxicity
AHH	aryl hydrocarbon hydroxylase
AMØ	alveolar macrophage
AO	acridine orange
APC	antigen presenting cell
BAC	bronchoalveolar carcinoma
BAL	bronchiol alveolar lavage
BALT	bronchial associated lymphoid tissue
BaP	benzo(a)pyrene
BSA	bovine serum albumin
CO_2	carbon dioxide
COPD	chronic obstructive pulmonary disease
CSI	cytoplamic staining intensity
Cyclo	cyclosporine
CV	crystal violet
DA_2PL+CPV	distemper adenovirus type 2 parainfluenza leptospira + canine parvo virus
DAB	diaminobenzidin
Dm	capillary membrane
DMBA	dimethylbenz(a)pyrene
DNA	deoxyribonucleic acid
DTNB	dithio-bi-nitrobenzoic acid
ELISA	enzyme link immunosorbent assay
EM	electron microscope
ERV	expiratory reserve volume
FCS	fraction of cytoplasmic staining
FRV	functional reserve volume

FEV	force expiratory volume
FEV_1	force expiratory volume in one second
FPN	fraction of positive nuclei
FPC	fraction of positive cytoplasm
GSH-Px	glutathione peroxidase
GST	glutathione S-transferase
GSTM1	glutathione S-transferase M1
LAL	left apical lobe
LALN	lung associated lymph node
LCL	left cardiac lobe
LDL	left diaphragmatic lobe
H & E	haematoxylin & Eosin
H_2O_2	hydrogen peroxide
Ig	immunoglobulin
IL	Interleukin
IL-2R	Interleukin 2 receptor
ILDS	interstitial lung disease
IRV	inspiratory reserve volume
NAC	n-acetylcysteine
NCLC	non-small cell lung cancer
NO	oxide of nitrogen
NRC	National Research Council
NSI	nuclear staining intensity
PAH	polycyclic aromatic hydrocarbon
PAS	Periodic Acid-Schiff
PBS	phosphate buffer saline
PCNA	proliferating cell nuclear antigen
PCO_2	partial pressure of carbon dioxide
PCR	polymerase chain reaction
PM	particulate matter
RAL	right apical lobe

RacL	right accessory lobe
RBC	red blood cell
RCL	right cardiac lobe
RDA	recommended daily allowance
RDL	right diaphragmatic lobe
RR	respiratory rate
RV	residual volume
rhIL2	recombinat human Interleukin 2
S	sulphur
SCLC	small cell lung cancer
SD	standard deviation
Se	selenium
SO_2	sulphur dioxide
SPM	suspended particulate matter
SRBC	sheep red blood cell
Th	T helper
TLC	total lung capacity
Tri	tricaprylin
VC	vital capacity
Vm	minute ventilation
Vt	tidal volume
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Air Pollution

Over centuries, the human population is very much concerned about air pollution, primarily in occupational settings and outdoors in urban areas that are mostly derived from automobile exhaust, industrial smoke and mines. Recently, air pollution becomes an important issue since its tremendous impacts are global, not only to animal and human health, but also to the economy of a nation. Nowadays, the major factor contributing to the air pollution phenomena termed 'haze', is large scale open burning of forest. It usually happened during recultivation in dry season for example at Kalimantan and Sumatra, Indonesia. Air pollutant components originating from biomass burning includes particulate matter (PM), polycyclic aromatic hydrocarbon (PAH), sulfur dioxide (SO_2), oxides of nitrogen (NO) and formaldehyde (Usmani *et al.*, 1998).

Haze

Haze is defined as suspended particles that are dispersed through a portion of the atmosphere. It is invisible to the naked eye and will grow in size as humidity increases.

The formation of a haze layer requires a source of haze particles and a relatively stable atmospheric condition in the lower layer of the atmosphere.

Atmospheric air PM with an aerodynamic diameter of 2.5 μm and less, and 2.5 μm - 10 μm is defined as PM2.5 and PM10, respectively. A very significant increase in the atmospheric PM concentration, particularly the finest particle was observed during the haze which covered the Malaysia atmosphere from July to December 1997 (Khalid *et al*., 1998).

Polycyclic aromatic hydrocarbon (PAH), which exists as colourless, white or pale yellow-green solids as a result of combustion and pyrolysis of organic substances is traditionally associated with PM includes benzo(a)pyrene (BaP), benzo(a)anthracene, pyrene, which may contribute to deleterious short and long-term health effects in human as well as animals.

Haze Episodes in Malaysia

The visibility depends on suspended particulate matter (SPM), particle sizes and relative humidity. Slight hazy conditions are common in Malaysia with visibility often below than 10 km, especially during the period of August - September. The hazy situation becomes worst when the visibility range reaches to < 1 km.