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

HAZILA WATI HAMZAH, D. V.M

FPV 2000 3
THE EFFECT OF BENZO(a)PYRENE (BAP) ON THE RESPIRATORY TRACT OF DOGS

By

HAZILAWATI HAMZAH, D.V.M

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Veterinary Science in the Faculty of Veterinary Medicine
Universiti Putra Malaysia

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, Ayo, 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.

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

By

HAZILAWATI HAMZAH, D. V. M.

September 2000

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 μg/dog intratracheally twice, six week apart, Se 20 μg/dog/day and cyclosporine at the dose 50 mg/m². 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 bronchoalveolar 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 ng 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

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September 2000

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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 untuk 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 μg/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 pembentukan barah paru-paru secara kasar dan mikroskopi, yang mana telah didiagnosis sebagai adenokarsinoma pulmonari dengan kemunculan protein 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 mempunyai 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 ng/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.
ACKNOWLEDGMENTS

I am especially grateful to my supervisors, Dr. Noordin Mohamed Mustapha, Prof. Dato' Dr. Sheikh Omar Abdul Rahman, and Dr. Daud Ahmad Israf Ali for their help in many ways and for their consistent advice, encouragement, moral support and excellent supervision throughout the course of the study.

I am also particularly grateful to Dr. Panayiotis Loukopoulos, Dr. Ng Kok Han, Mrs. Nor Azura Salim, Mrs. Azlina Mohd Salim, Mrs. Hartina Abdul Khan, Mr. Hari Govindan, Mrs. Azimah and Mrs. Sakdiah for their kind guidance in immunology and special staining technique. Thank is also extended to the excellent help of Tuan Haji Mohamad Nor and Mr. Jamil for their guidance in processing and preparation of histology specimens. Special thanks to Dr. Shizhen Zhang, Dr. Thoria, Dr. Muthafar, Dr. Goh Yong Meng, Mr. Ghazali Yusof, Mr. Noraziman Sulaiman, Mr. Apparao a/l Somanaidu, Chamdre a/l Vengadasamy, Miss. Maizatul Akmal Moktar, all the staff of the Faculty of Veterinary Medicine, Universiti Putra Malaysia (U.P.M) for their technical support and to all the staff of the Dog Unit, Dewan Bandaraya Kuala Lumpur, for providing healthy dogs for this study.

Sincere gratitude is also conveyed to the Ministry of Science, Technology and the Environment of Malaysia for the provision of the IRPA grant (06-02-04-0071) and the National Sciences Fellowship for the scholarship.
Last but not least, the consistent moral and technical support, patience and understanding of my loving husband, Dr. Mohd Rosly Shaari throughout the course of the study will always be remembered and appreciated.
I certify that an Examination Committee met on 26th September 2000 to conduct the final examination of Hazilawati Binti Hamzah on her Master of Veterinary Science thesis entitled "The Study of the Effect of Benzo(a)pyrene (BaP) on the Respiratory Tract of Dogs" 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:

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Date: 11 JAN 2001
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.

Date: 27 NOV 2000

Hazlizawati Hamzah, D.V.M.
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<td>atypical adenomatous hyperplasia</td>
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<td>ADCC</td>
<td>antibody-dependent cellular cytotoxicity</td>
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<tr>
<td>AHH</td>
<td>aryl hydrocarbon hydroxylase</td>
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<td>AM0</td>
<td>alveolar macrophage</td>
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<tr>
<td>AO</td>
<td>acridine orange</td>
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<tr>
<td>APC</td>
<td>antigen presenting cell</td>
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<tr>
<td>BAC</td>
<td>bronchoalveolar carcinoma</td>
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<td>BAL</td>
<td>bronchiol alveolar lavage</td>
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<td>BALT</td>
<td>bronchial associated lymphoid tissue</td>
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<td>BaP</td>
<td>benzo(a)pyrene</td>
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<td>BSA</td>
<td>bovine serum albumin</td>
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<td>CO₂</td>
<td>carbon dioxide</td>
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<td>COPD</td>
<td>chronic obstructive pulmonary disease</td>
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<td>CSI</td>
<td>cytoplasmic staining intensity</td>
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<td>Cyclo</td>
<td>cyclosporine</td>
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<td>CV</td>
<td>crystal violet</td>
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<td>DA₂PL+CPV</td>
<td>distemper adenovirus type 2 parainfluenza leptospira + canine parvo virus</td>
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<td>DAB</td>
<td>diaminobenzidin</td>
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<td>Dm</td>
<td>capillary membrane</td>
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<td>DMBA</td>
<td>dimethylbenz(a)pyrene</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>DTNB</td>
<td>dithio-bi-nitrobenzoic acid</td>
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<td>ELISA</td>
<td>enzyme link immunosorbent assay</td>
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<tr>
<td>EM</td>
<td>electron microscope</td>
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<tr>
<td>ERV</td>
<td>expiratory reserve volume</td>
</tr>
<tr>
<td>FCS</td>
<td>fraction of cytoplasmic staining</td>
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<td>FRV</td>
<td>functional reserve volume</td>
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FEV  force expiratory volume
FEV₁  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
H₂O₂  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
PCO₂  partial pressure of carbon dioxide
PCR  polymerase chain reaction
PM  particulate matter
RAL  right apical lobe
<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>RacL</td>
<td>right accessory lobe</td>
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<tr>
<td>RBC</td>
<td>red blood cell</td>
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<td>RCL</td>
<td>right cardiac lobe</td>
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<td>RDA</td>
<td>recommended daily allowance</td>
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<td>RDL</td>
<td>right diaphragmatic lobe</td>
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<td>RR</td>
<td>respiratory rate</td>
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<td>RV</td>
<td>residual volume</td>
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<td>rhIL2</td>
<td>recombinant human Interleukin 2</td>
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<td>S</td>
<td>sulphur</td>
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<td>SCLC</td>
<td>small cell lung cancer</td>
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<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>Se</td>
<td>selenium</td>
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<tr>
<td>SO₂</td>
<td>sulphur dioxide</td>
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<td>SPM</td>
<td>suspended particulate matter</td>
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<td>SRBC</td>
<td>sheep red blood cell</td>
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<td>Th</td>
<td>T helper</td>
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<tr>
<td>TLC</td>
<td>total lung capacity</td>
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<tr>
<td>Tri</td>
<td>tricaprylin</td>
</tr>
<tr>
<td>VC</td>
<td>vital capacity</td>
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<tr>
<td>Vm</td>
<td>minute ventilation</td>
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<td>Vt</td>
<td>tidal volume</td>
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<td>WHO</td>
<td>World Health Organization</td>
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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.